PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68

A2

(11) International Publication Number: **WO 99/54500**

(43) International Publication Date: 28 October 1999 (28.10.99)

(21) International Application Number: PCT/IB99/00822

(22) International Filing Date: 21 April 1999 (21.04.99)

(30) Priority Data:

60/082,614	21 April 1998 (21.04.98)	US
60/109,732	23 November 1998 (23.11.98)	US

(71) Applicant *(for all designated States except US)*: GENSET [FR/FR]; 24, rue Royale, F–75008 Paris (FR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: COHEN, Daniel [FR/FR]; 5, avenue Odette, F–94120 Fontenay–sous–Bois (FR). BLUMENFELD, Marta [FR/FR]; 5, rue Tagore, F–75013 Paris (FR). CHUMAKOV, Ilya [FR/FR]; 196, rue des Chèvrefeuilles, F–77000 Vaux–le–Pénil (FR).

(74) Agents: MARTIN Jean–Jacques et al.; Cabinet Regimbeau, 26, avenue Kléber, F–75116 Paris (FR).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: BIALLELIC MARKERS FOR USE IN CONSTRUCTING A HIGH DENSITY DISEQUILIBRIUM MAP OF THE HUMAN GENOME

(57) Abstract

The present invention relates to genomic maps comprising biallelic markers, new biallelic markers, and methods of using biallelic markers. Primers hybridizing to regions flanking these biallelic markers are also provided. This invention provides polynucleotides and methods suitable for genotyping a nucleic acid containing sample for one or more biallelic markers of the invention. Further, the invention provides a number of methods utilizing the biallelic markers of the invention including methods to detect a statistical correlation between a biallelic marker allele and a phenotype and/or between a biallelic marker haplotype and a phenotype.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

BIALLELIC MARKERS FOR USE IN CONSTRUCTING A HIGH DENSITY DISEQUILIBRIUM MAP OF THE HUMAN GENOME

Background of the Invention

Recent advances in genetic engineering and bioinformatics have enabled the manipulation and characterization of large portions of the human genome. While efforts to obtain the full sequence of the human genome are rapidly progressing, there are many practical uses for genetic information which can be implemented with partial knowledge of the sequence of the human genome.

As the full sequence of the human genome is assembled, the partial sequence information available can be used to identify genes responsible for detectable human traits, such as genes associated with human diseases, and to develop diagnostic tests capable of identifying individuals who express a detectable trait as the result of a specific genotype or individuals whose genotype places them at risk of developing a detectable trait at a subsequent time. Each of these applications for partial genomic sequence information is based upon the assembly of genetic and physical maps which order the known genomic sequences along the human chromosomes.

The present invention relates to an ordered set of human genomic sequences comprising single nucleotide polymorphisms, as well as the use of these polymorphisms as a high resolution map of the human genome, methods of identifying genes associated with detectable human traits, and diagnostics for identifying individuals who carry a gene which causes them to express a detectable trait or which places them at risk of expressing a detectable trait in the future.

Advantages of the biallelic markers of the present invention

The map-related biallelic markers of the present invention offer a number of important advantages over other genetic markers such as RFLP (Restriction fragment length polymorphism), VNTR (Variable Number of Tandem Repeats) markers and earlier STS-(sequence tagged sites) derived markers.

The first generation of markers, were RFLPs, which are variations that modify the length of a restriction fragment. But methods used to identify and to type RFLPs are relatively wasteful of materials, effort, and time. Since they are biallelic markers (they present only two alleles, the restriction site being either present or absent), their maximum heterozygosity is 0.5. The theoretical number of RFLPs distributed along the entire human genome is more than 10^5, which leads to a potential average inter-marker distance of 30 kilobases. However,

in reality the number of evenly distributed RFLPs which occur at a sufficient frequency in the population to make them useful for tracking of genetic polymorphisms is very limited.

The second generation of genetic markers were VNTRs, which can be categorized as either minisatellites or microsatellites. Minisatellites are tandemly repeated DNA sequences present in units of 5-50 repeats which are distributed along regions of the human chromosomes ranging from 0.1 to 20 kilobases in length. Since they present many possible alleles, their informative content is very high. Minisatellites are scored by performing Southern blots to identify the number of tandem repeats present in a nucleic acid sample from the individual being tested. However, there are only 10^4 potential VNTRs that can be typed by Southern blotting. Thus, the number of easily typed informative markers in these maps is far too small for the average distance between informative markers to fulfill the requirements for a useful genetic map. Moreover, both RFLP and VNTR markers are costly and time-consuming to develop and assay in large numbers.

Initial attempts to construct genetic maps based on non-RFLP biallelic markers have focused on identifying biallelic markers lying within sequence tagged sites (STS), pieces of genomic DNA having a known sequence and averaging about 250 bases in length. More than 30,000 STSs have been identified and ordered along the genome (Hudson et al., *Science* 270:1945-1954 (1995); Schuler et al., *Science* 274:540-546 (1996)). For example, the Whitehead Institute and Généthon's integrated map contains 15,086 STSs.

These sequence tagged sites can be screened to identify polymorphisms, preferably Single Nucleotide Polymorphisms (SNPs), more preferably non RFLP biallelic markers therein. Generally polymorphisms are identified by determining the sequence of the STSs in 5 to 10 individuals.

Wang et al. (Cold Spring harbor laboratory: *Abstracts of papers presented on genome Mapping and sequencing* p.17 (May 14-18, 1997)) recently announced the identification and mapping of 750 Single Nucleotide Polymorphisms issued from the sequencing of 12,000 STSs from the Whitehead/MIT map, in eight unrelated individuals. The map was assembled using a high throughput system based on the utilization of DNA chip technology available from Affymetrix (Chee et al., *Science* 274:610-614 (1996)).

However, according to experimental data and statistical calculations, less than one out of 10 of all STSs mapped today will contain an informative Single Nucleotide Polymorphism. This is primarily due to the short length of existing STSs (usually less than 250 bp). If one assumes 10^6 informative SNPs spread along the human genome, there would on average be one marker of interest every $3X10^9/10^6$, i.e. every 3,000 bp. The probability that one such marker is present on a 250 bp stretch is thus less than 1/10.

While it could produce a high density map, the STS approach based on currently existing markers does not put any systematic effort into making sure that the markers obtained are optimally distributed throughout the entire genome. Instead, polymorphisms are limited to those locations for which STSs are available.

The even distribution of markers along the chromosomes is critical to the future success of genetic analyses. In particular, a high density map having appropriately spaced markers is essential for conducting association studies on sporadic cases, aiming at identifying genes responsible for detectable traits such as those which are described below.

As will be further explained below, genetic studies have mostly relied in the past on a statistical approach called linkage analysis, which took advantage of microsatellite markers to study their inheritance pattern within families from which a sufficient number of individuals presented the studied trait. Because of intrinsic limitations of linkage analysis, which will be further detailed below, and because these studies necessitate the recruitment of adequate family pedigrees, they are not well suited to the genetic analysis of all traits, particularly those for which only sporadic cases are available (e.g. drug response traits), or those which have a low penetrance within the studied population.

Association studies enabled by the biallelic markers of the present invention offer an alternative to linkage analysis. Combined with the use of a high density map of appropriately spaced, sufficiently informative markers, association studies, including linkage disequilibrium-based genome wide association studies, will enable the identification of most genes involved in complex traits.

Single nucleotide polymorphism or biallelic markers can be used in the same manner as RFLPs and VNTRs but offer several advantages. Single nucleotide polymorphisms are densely spaced in the human genome and represent the most frequent type of variation. An estimated number of more than 10^7 sites are scattered along the $3x10^9$ base pairs of the human genome. Therefore, single nucleotide polymorphisms occur at a greater frequency and with greater uniformity than RFLP or VNTR markers which means that there is a greater probability that such a marker will be found in close proximity to a genetic locus of interest. Single nucleotide polymorphisms are less variable than VNTR markers but are mutationally more stable.

Also, the different forms of a characterized single nucleotide polymorphism, such as the biallelic markers of the present invention, are often easier to distinguish and can therefore be typed easily on a routine basis. Biallelic markers have single nucleotide based alleles and they have only two common alleles, which allows highly parallel detection and automated scoring. The biallelic markers of the present invention offer the possibility of rapid, high-throughput genotyping of a large number of individuals.

Biallelic markers are densely spaced in the genome, sufficiently informative and can be assayed in large numbers. The combined effects of these advantages make biallelic markers extremely valuable in genetic studies. Biallelic markers can be used in linkage studies in families, in allele sharing methods, in linkage disequilibrium studies in populations, in association studies of case-control populations. An important aspect of the present invention is that biallelic markers allow association studies to be performed to identify genes involved in complex traits. Association studies examine the frequency of marker alleles in unrelated case- and control-populations and are generally employed in the detection of polygenic or sporadic traits. Association studies may be conducted within the general population and are not limited to studies performed on related individuals in affected families (linkage studies). Biallelic markers in different genes can be screened in parallel for direct association with disease or response to a treatment. This multiple gene approach is a powerful tool for a variety of human genetic studies as it provides the necessary statistical power to examine the synergistic effect of multiple genetic factors on a particular phenotype, drug response, sporadic trait, or disease state with a complex genetic etiology.

The present invention relates to a high density linkage disequilibrium-based genetic maps of the human genome which comprise the map-related biallelic markers of the invention and will allow the identification of genes responsible for detectable traits using genome-wide association studies and linkage disequilibrium mapping.

Summary of the Invention

The present invention is based on the discovery of a set of novel map-related biallelic markers. See Table 1. The position of these markers and knowledge of the surrounding sequence has been used to design polynucleotide compositions which are useful in high density mapping of the human genome as well as in determining the identity of nucleotides at the marker position, and more complex association and haplotyping studies which are useful in determining the genetic basis for disease states. In addition, the compositions and methods of the invention find use in the identification of the targets for the development of pharmaceutical agents and diagnostic methods, as well as the characterization of the differential efficacious responses to and side effects from pharmaceutical agents acting on a disease as well as other treatments.

A first embodiment of the present invention is a map of the human genome comprising an ordered array of biallelic markers, wherein at least 1, 2, 5, 10, 20, 25, 30, 50, 100, 200, 500, 1000, 2000 or 3000 of said biallelic markers are map-related biallelic markers. In addition, the maps of the present invention encompass maps with any further limitation described in this disclosure, or those following, specified alone or in any combination:

optionally, said map-related biallelic marker may be selected individually or in any combination from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally said ordered array comprises at least 20,000, 40,000, 60,000, 80,000, 100,000, or 120,000 biallelic markers; optionally, wherein said biallelic markers are separated from one another by an average distance of 10kb-200 kb, 15kb-150 kb, 20kb-100 kb, 100kb-150 kb, 50-100kb, or 25 kb-50 kb in the human genome; optionally, said biallelic markers are distributed at an average density of at least one biallelic marker every 150kb, 50 kb, or 30 kb in the human genome; or optionally, wherein, all of said biallelic markers are selected to have a heterozygosity rates of at least about 0.18, 0.32, or 0.42.

A second embodiment of the invention encompasses isolated, purified or recombinant polynucleotides consisting of, consisting essentially of, or comprising a contiguous span of nucleotides of a sequence selected as an individual or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908, 3935 to 7842, 7866 to 11773, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 10125, 10126 to 11599, and 11600 to 11773, or the complements thereof, wherein said contiguous span is at least 8, 10, 12, 15, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID. The present invention also relates to polynucleotides hybridizing under stringent or intermediate conditions to a sequence selected from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908, 3935 to 7842, 7866 to 11773, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 10125, 10126 to 11599, and 11600 to 11773 and the complements thereof. In addition, the polynucleotides of the invention encompass polynucleotides with any further limitation described in this disclosure, or those following, specified alone or in any combination: said contiguous span may optionally comprise a map-related biallelic marker; optionally either the 1ST or the 2ND allele of the respective SEQ ID No., as indicated in Table 1, may be specified as being present at said map-related biallelic marker; optionally, said biallelic marker may be within 6, 5, 4, 3, 2, or 1 nucleotides of the center of said polynucleotide or at the center of said polynucleotide; optionally, said polynucleotide may consists of, or consist essentially of a contiguous span which ranges in length from 8, 10, 12, 15, 18 or 20 to 21, 25, 35, 40, 43, or 47 nucleotides; optionally, said polynucleotide may consists of, or consist essentially of a contiguous span which ranges in length from 8, 10, 12, 15, 18 or 20 to 21, 25, 35, 40, 43, or 47 nucleotides, or be specified as being 12, 15, 18, 20, 25, 35, 40, 43, or 47 nucleotides in length and including an map-related biallelic marker of said sequence, and optionally the 1st allele of Table 1 is present at said biallelic marker; optionally, the 3' end of said contiguous span may be present

at the 3' end of said polynucleotide; optionally, biallelic marker may be present at the 3' end of said polynucleotide; optionally, the 3' end of said polynucleotide may be located within or at least 2, 4, 6, 8, or 10 nucleotides upstream of a map-related biallelic marker in said sequence, to the extent that such a distance is consistent with the lengths of the particular Sequence ID; optionally, the 3' end of said polynucleotide may be located 1 nucleotide upstream of a map-related biallelic marker in said sequence; and optionally, said polynucleotide may further comprise a label.

A third embodiment of the invention encompasses any polynucleotide of the invention attached to a solid support. In addition, the polynucleotides of the invention which are attached to a solid support encompass polynucleotides with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said polynucleotides may be specified as attached individually or in groups of at least 2, 5, 8, 10, 12, 15, 20, 25, 50, 100, 200, or 500 distinct polynucleotides of the inventions to a single solid support; optionally, polynucleotides other than those of the invention may attached to the same solid support as polynucleotides of the invention; optionally, when multiple polynucleotides are attached to a solid support they may be attached at random locations, or in an ordered array; optionally, said ordered array may be addressable.

A fourth embodiment of the invention encompasses the use of any polynucleotide for, or any polynucleotide for use in, determining the identity of nucleotides at a map-related biallelic marker. In addition, the polynucleotides of the invention for use in determining the identity of nucleotides at a map-related biallelic marker encompass polynucleotides with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be selected individually or in any combination from the group consisting of the biallelic markers of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, said polynucleotide may comprise a sequence disclosed in the present specification; optionally, said polynucleotide may consist of, or consist essentially of any polynucleotide described in the present specification; optionally, said determining may be performed in a hybridization assay, sequencing assay, microsequencing assay, or an enzyme-based mismatch detection assay; optionally, said polynucleotide may be attached to a solid support, array, or addressable array; optionally, said polynucleotide may be labeled.

A fifth embodiment of the invention encompasses the use of any polynucleotide for, or any polynucleotide for use in, amplifying a segment of nucleotides comprising a map-related biallelic marker. In addition, the polynucleotides of the invention for use in amplifying a segment of nucleotides comprising a map-related biallelic marker encompass polynucleotides with any further limitation described in this disclosure, or those following,

specified alone or in any combination: optionally, said map-related biallelic marker may be selected individually or in any combination from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, said polynucleotide may consist of, consist essentially of, or comprise a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 3935 to 7842, 7866 to 11773, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 10125, 10126 to 11599, and 11600 to 11773; optionally, said polynucleotide may consist of, or consist essentially of any polynucleotide described in the present specification; optionally, said amplifying may be performed by a PCR or LCR. Optionally, said polynucleotide may be attached to a solid support, array, or addressable array. Optionally, said polynucleotide may be labeled.

A sixth embodiment of the invention encompasses methods of genotyping a biological sample comprising determining the identity of a nucleotide at a map-related biallelic marker. In addition, the genotyping methods of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be selected individually or in any combination from the group consisting of the biallelic markers of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, said method further comprises determining the identity of a second nucleotide at said biallelic marker, wherein said first nucleotide and second nucleotide are not base paired (by Watson & Crick base pairing) to one another; optionally, said biological sample is derived from a single individual or subject; optionally, said method is performed *in vitro*; optionally, said biallelic marker is determined for both copies of said biallelic marker present in said individual's genome; optionally, said biological sample is derived from multiple subjects or individuals; optionally, said method further comprises amplifying a portion of said sequence comprising the biallelic marker prior to said determining step; optionally, wherein said amplifying is performed by PCR, LCR, or replication of a recombinant vector comprising an origin of replication and said portion in a host cell; optionally, wherein said determining is performed by a hybridization assay, sequencing assay, microsequencing assay, or an enzyme-based mismatch detection assay.

A seventh embodiment of the invention comprises methods of estimating the frequency of an allele in a population comprising genotyping individuals from said population for a map-related biallelic marker and determining the proportional representation of said biallelic marker in said population. In addition, the methods of estimating the frequency of an allele in a population of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination:

optionally, said map-related biallelic marker may be selected individually or in any combination from the group consisting of the biallelic markers of SEQ Nos. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, determining the frequency of a biallelic marker allele in a population may be accomplished by determining the identity of the nucleotides for both copies of said biallelic marker present in the genome of each individual in said population and calculating the proportional representation of said nucleotide at said map-related biallelic marker for the population; optionally, determining the frequency of a biallelic marker allele in a population may be accomplished by performing a genotyping method on a pooled biological sample derived from a representative number of individuals, or each individual, in said population, and calculating the proportional amount of said nucleotide compared with the total.

An eighth embodiment of the invention comprises methods of detecting an association between an allele and a phenotype, comprising the steps of a) determining the frequency of at least one map-related biallelic marker allele in a trait positive population, b) determining the frequency of said map-related biallelic marker allele in a control population and; c) determining whether a statistically significant association exists between said genotype and said phenotype. In addition, the methods of detecting an association between an allele and a phenotype of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be selected individually or in any combination from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, said control population may be a trait-negative population, or a random population; optionally, wherein said phenotype is selected from the group consisting of disease, treatment response, treatment efficacy, drug response, drug efficacy, and drug toxicity; optionally, the determining steps a) and b) are performed on all of the biallelic markers of SEQ ID Nos. 1 to 3908.

An ninth embodiment of the present invention encompasses methods of estimating the frequency of a haplotype for a set of biallelic markers in a population, comprising the steps of: a) genotyping each individual in said population for at least one map-related biallelic marker, b) genotyping each individual in said population for a second biallelic marker by determining the identity of the nucleotides at said second biallelic marker for both copies of said second biallelic marker present in the genome; and c) applying a haplotype determination method to the identities of the nucleotides determined in steps a) and b) to obtain an estimate of said frequency. In addition, the methods of estimating the frequency of a haplotype of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally said haplotype determination

method is selected from the group consisting of asymmetric PCR amplification, double PCR amplification of specific alleles, the Clark method, or an expectation maximization algorithm; optionally, said map-related biallelic marker may be selected individually or in any combination from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, said second biallelic marker is a map-related biallelic marker; optionally, the identity of the nucleotides at the biallelic markers in every one of the sequences of SEQ ID No. 1 to 3908 is determined in steps a) and b).

A tenth embodiment of the present invention encompasses methods of detecting an association between a haplotype and a phenotype, comprising the steps of: a) estimating the frequency of at least one haplotype in a trait positive population according to a method of estimating the frequency of a haplotype of the invention; b) estimating the frequency of said haplotype in a control population according to the method of estimating the frequency of a haplotype of the invention; and c) determining whether a statistically significant association exists between said haplotype and said phenotype. In addition, the methods of detecting an association between a haplotype and a phenotype of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, said control population may be a trait-negative population, or a random population; optionally, wherein said phenotype is selected from the group consisting of disease, treatment response, treatment efficacy, drug response, drug efficacy, and drug toxicity; optionally, the identity of the nucleotides at the biallelic markers in every one of the following sequences: SEQ ID No. 1 to 3908 is included in the estimating steps a) and b).

An eleventh embodiment of the present invention is a method of identifying a gene associated with a detectable trait comprising the steps of: a) determining the frequency of each allele of at least one map-related biallelic marker in individuals having the detectable trait and individuals lacking the detectable trait; b) identifying at least one alleles of one or biallelic markers having a statistically significant association with the detectable trait; and c) identifying a gene in linkage disequilibrium with said allele. In addition, the methods of the present invention for identifying a gene associated with a detectable trait encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, wherein the method further comprises d) identifying a mutation in the gene identified in step c) which is associated with the detectable trait; optionally, wherein the individuals having the detectable trait and the individuals lacking the

detectable trait are readily distinguishable from one another; optionally, wherein the individuals having the detectable trait and the individuals lacking the detectable trait are selected from a bimodal population; optionally, wherein the individuals having the detectable trait are at one extreme of the population and the individuals lacking the detectable trait are at the other extreme of the population; optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, wherein said detectable trait is selected from the group consisting of disease, treatment response, treatment efficacy, drug response, drug efficacy, and drug toxicity.

A twelfth embodiment of the present invention is a method of identifying biallelic markers associated with a detectable trait comprising the steps of: a) determining the frequencies of a set of biallelic markers comprising at least one map-related biallelic marker in individuals who express said detectable trait and individuals who do not express said detectable trait; and b) identifying one or more biallelic markers in said set which are statistically associated with the expression of said detectable trait. In addition, the methods of the present invention for identifying biallelic markers associated with a detectable trait encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, wherein said detectable trait is selected from the group consisting of disease, treatment response, treatment efficacy, drug response, drug efficacy, and drug toxicity.

A thirteenth embodiment of the present invention is a method of identifying biallelic marker(s) in linkage disequilibrium with a trait causing allele or in linkage disequilibrium with a trait-associated biallelic marker comprising the steps of: a) selecting at least one map-related biallelic marker which is in the genomic region suspected of containing the trait-causing allele or the trait-associated biallelic marker; and b) determining which of the map-related biallelic markers are associated with the trait-causing allele or in linkage disequilibrium with the trait-associated biallelic marker. In addition, the methods of the present invention for identifying biallelic marker(s) in linkage disequilibrium with a trait causing allele or in linkage disequilibrium with a trait-associated biallelic marker encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, wherein said

detectable trait is selected from the group consisting of disease, treatment response, treatment efficacy, drug response, drug efficacy, and drug toxicity.

A fourteenth embodiment of the present invention is a method for determining whether an individual is at risk of developing a detectable trait or suffers from a detectable trait comprising the steps of: a) obtaining a nucleic acid sample from the individual; b) screening the nucleic acid sample with at least one map-related biallelic marker; and c) determining whether the nucleic acid sample contains at least one allele of said map-related biallelic marker statistically associated with the detectable trait. In addition, the methods of the present invention for determining whether an individual is at risk of developing a detectable trait or suffers from a detectable trait encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, wherein said detectable trait is selected from the group consisting of disease, treatment response, treatment efficacy, drug response, drug efficacy, and drug toxicity.

A fifteenth embodiment of the present invention is a method of administering a drug or a treatment comprising the steps of: a) obtaining a nucleic acid sample from an individual; b) determining the identity of the polymorphic base of at least one map-related biallelic marker which is associated with a positive response to the treatment or the drug; or at least one biallelic map-related marker which is associated with a negative response to the treatment or the drug; and c) administering the treatment or the drug to the individual if the nucleic acid sample contains said biallelic marker associated with a positive response to the treatment or the drug or if the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug. In addition, the methods of the present invention for administering a drug or a treatment encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; or optionally, the administering step comprises administering the drug or the treatment to the individual if the nucleic acid sample contains said biallelic marker associated with a positive response to the treatment or the drug and the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug.

A sixteenth embodiment of the present invention is a method of selecting an individual for inclusion in a clinical trial of a treatment or drug comprising the steps of: a)

obtaining a nucleic acid sample from an individual; b) determining the identity of the polymorphic base of at least one map-related biallelic marker which is associated with a positive response to the treatment or the drug, or at least one map-related biallelic marker which is associated with a negative response to the treatment or the drug in the nucleic acid sample, and c) including the individual in the clinical trial if the nucleic acid sample contains said map-related biallelic marker associated with a positive response to the treatment or the drug or if the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug. In addition, the methods of the present invention for selecting an individual for inclusion in a clinical trial of a treatment or drug encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, the including step comprises administering the drug or the treatment to the individual if the nucleic acid sample contains said biallelic marker associated with a positive response to the treatment or the drug and the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug.

A seventeenth embodiment of the present invention is a method of identifying a gene associated with a detectable trait comprising the steps of: a) selecting a gene suspected of being associated with a detectable trait; and b) identifying at least one map-related biallelic marker within said gene which is associated with said detectable trait. In addition, the methods of the present invention for identifying a gene associated with a detectable trait encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said map-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3734, 3734 to 3908 and the complements thereof; optionally, the identifying step comprises determining the frequencies of the map-related biallelic marker(s) in individuals who express said detectable trait and individuals who do not express said detectable trait and identifying one or more biallelic markers which are statistically associated with the expression of the detectable trait.

Additional embodiments are set forth in the Detailed Description of the Invention and in the Examples.

Brief Description of the Drawings

Figure 1 is a cytogenetic map of chromosome 21.

Figure 2a shows the results of a computer simulation of the distribution of inter-marker spacing on a randomly distributed set of biallelic markers indicating the percentage of biallelic markers which will be spaced a given distance apart for 1, 2, or 3 markers/BAC in a genomic map (assuming a set of 20,000 minimally overlapping BACs covering the genome are evaluated).

Figure 2b shows the results of a computer simulation of the distribution of inter-marker spacing on a randomly distributed set of biallelic markers indicating the percentage of biallelic markers which will be spaced a given distance apart for 1, 3, or 6 markers/BAC in a genomic map (assuming a set of 20,000 minimally overlapping BACs covering the genome are evaluated).

Figure 3 shows, for a series of hypothetical sample sizes, the p-value significance obtained in association studies performed using individual markers from the high-density biallelic map, according to various hypotheses regarding the difference of allelic frequencies between the trait-positive and trait-negative samples.

Figure 4 is a hypothetical association analysis conducted with a map comprising about 3,000 biallelic markers.

Figure 5 is a hypothetical association analysis conducted with a map comprising about 20,000 biallelic markers.

Figure 6 is a hypothetical association analysis conducted with a map comprising about 60,000 biallelic markers.

Figure 7 is a haplotype analysis using biallelic markers in the Apo E region.

Figure 8 is a simulated haplotype analysis using the biallelic markers in the Apo E region included in the haplotype analysis of Figure 7.

Figure 9 shows a minimal array of overlapping clones which was chosen for further studies of biallelic markers associated with prostate cancer, the positions of STS markers known to map in the candidate genomic region along the contig, and the locations of biallelic markers along the BAC contig harboring a genomic region harboring a candidate gene associated with prostate cancer which were identified using the methods of the present invention.

Figure 10 is a rough localization of a candidate gene for prostate cancer which was obtained by determining the frequencies of the biallelic markers of Figure 9 in affected and unaffected populations.

Figure 11 is a further refinement of the localization of the candidate gene for prostate cancer using additional biallelic markers which were not included in the rough localization illustrated in Figure 10.

Figure 12 is a haplotype analysis using the biallelic markers in the genomic region of the gene associated with prostate cancer.

Figure 13 is a simulated haplotype using the six markers included in haplotype 5 of Figure 12.

Figure 14 is a block diagram of an exemplary computer system.

Figure 15 is a flow diagram illustrating one embodiment of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database.

Figure 16 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous.

Figure 17 is a flow diagram illustrating one embodiment of an identifier process 300 for detecting the presence of a feature in a sequence.

Brief Description Of The Sequence Listing

SEQ ID Nos. 1 to 3908 contain nucleotide sequences comprising a portion of the map-related biallelic markers of the invention.

SEQ ID Nos. 3909 to 3934 contain nucleotide sequences comprising a portion of the map-related biallelic markers which are shown to be associated with Alzheimer's disease, prostate cancer or asthma as described in the Examples.

SEQ ID Nos. 3935 to 7842 contain nucleotide sequences of upstream amplification primers (PU) designed to amplify sequences containing the biallelic markers of SEQ ID Nos. 1 to 3908.

SEQ ID Nos. 7843 to 7865 contain nucleotide sequences of upstream amplification primers (PU) designed to amplify sequences containing the biallelic markers of SEQ ID Nos. 3909 to 3934.

SEQ ID Nos. 7866 to 11773 contain nucleotide sequences of downstream amplification primers (RP) designed to amplify sequences containing the biallelic markers of SEQ ID Nos. 1 to 3908.

SEQ ID Nos. 11774 to 11796 contain nucleotide sequences of downstream amplification primers (RP) designed to amplify sequences containing the biallelic markers of SEQ ID Nos. 3909 to 3934.

Detailed Description of the Embodiments

Before describing the invention in greater detail, the following definitions are set forth to illustrate and define the meaning and scope of the terms used to describe the invention herein.

Definitions

As used interchangeably herein, the terms "nucleic acids" "oligonucleotides", and "polynucleotides" include RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form. The term "nucleotide" as used herein as an adjective to describe molecules comprising RNA, DNA, or RNA/DNA hybrid sequences of any length in single-stranded or duplex form. The term "nucleotide" is also used herein as a noun to refer to individual nucleotides or varieties of nucleotides, meaning a molecule, or individual unit in a larger nucleic acid molecule, comprising a purine or pyrimidine, a ribose or deoxyribose sugar moiety, and a phosphate group, or phosphodiester linkage in the case of nucleotides within an oligonucleotide or polynucleotide. Although the term "nucleotide" is also used herein to encompass "modified nucleotides" which comprise at least one modifications (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar, for examples of analogous linking groups, purine, pyrimidines, and sugars see for example PCT publication No. WO 95/04064. However, the polynucleotides of the invention are preferably comprised of greater than 50% conventional deoxyribose nucleotides, and most preferably greater than 90% conventional deoxyribose nucleotides. The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, *ex vivo* generation, or a combination thereof, as well as utilizing any purification methods known in the art.

The term "purified" is used herein to describe a polynucleotide or polynucleotide vector of the invention which has been separated from other compounds including, but not limited to other nucleic acids, carbohydrates, lipids and proteins (such as the enzymes used in the synthesis of the polynucleotide), or the separation of covalently closed polynucleotides from linear polynucleotides. A polynucleotide is substantially pure when at least about 50 %, preferably 60 to 75% of a sample exhibits a single polynucleotide sequence and conformation (linear versus covalently close). A substantially pure polynucleotide typically comprises about 50 %, preferably 60 to 90% weight/weight of a nucleic acid sample, more usually about 95%, and preferably is over about 99% pure. Polynucleotide purity or homogeneity may be indicated by a number of means well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polynucleotide band upon staining the gel. For certain purposes higher resolution can be provided by using HPLC or other means well known in the art.

The term "primer" denotes a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase, RNA polymerase or reverse transcriptase.

The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, e.g., polynucleotide as defined herein) which can be used to identify a specific polynucleotide sequence present in samples, said nucleic acid segment comprising a nucleotide sequence complementary of the specific polynucleotide sequence to be identified.

The terms "detectable trait" "trait" and "phenotype" are used interchangeably herein and refer to any visible, detectable or otherwise measurable property of an organism such as symptoms of, or susceptibility to a disease for example. Typically the terms "detectable trait" "trait" or "phenotype" are used herein to refer to symptoms of, or susceptibility to a disease; or to refer to an individual's response to an agent, drug, or treatment acting on a disease; or to refer to symptoms of, or susceptibility to side effects to an agent acting on a disease.

The term "treatment" is used herein to encompass any medical intervention known in the art including, for example, the administration of pharmaceutical agents, medically prescribed changes in diet, or habits such as a reduction in smoking or drinking, surgery, the application of medical devices, and the application or reduction of certain physical conditions, for example, light or radiation.

The term "allele" is used herein to refer to variants of a nucleotide sequence. A biallelic polymorphism has two forms; designated herein as the 1^{ST} allele and the 2^{ND} allele. Diploid organisms may be homozygous or heterozygous for an allelic form.

The term "heterozygosity rate" is used herein to refer to the incidence of individuals in a population, which are heterozygous at a particular allele. In a biallelic system the heterozygosity rate is on average equal to $2P_a(1-P_a)$, where P_a is the frequency of the least common allele. In order to be useful in genetic studies a genetic marker should have an adequate level of heterozygosity to allow a reasonable probability that a randomly selected person will be heterozygous.

The term "genotype" as used herein refers the identity of the alleles present in an individual or a sample. In the context of the present invention a genotype preferably refers to the description of the biallelic marker alleles present in an individual or a sample. The term "genotyping" a sample or an individual for a biallelic marker consists of determining the specific allele or the specific nucleotide carried by an individual at a biallelic marker.

The term "mutation" as used herein refers to a difference in DNA sequence between or among different genomes or individuals which has a frequency below 1%.

The term "haplotype" refers to a combination of alleles present in an individual or a sample. In the context of the present invention a haplotype preferably refers to a combination of biallelic marker alleles found in a given individual and which may be associated with a phenotype.

The term "polymorphism" as used herein refers to the occurrence of two or more

alternative genomic sequences or alleles between or among different genomes or individuals. "Polymorphic" refers to the condition in which two or more variants of a specific genomic sequence can be found in a population. A "polymorphic site" is the locus at which the variation occurs. A single nucleotide polymorphism is a single base pair change. Typically a single nucleotide polymorphism is the replacement of one nucleotide by another nucleotide at the polymorphic site. Deletion of a single nucleotide or insertion of a single nucleotide, also give rise to single nucleotide polymorphisms. In the context of the present invention "single nucleotide polymorphism" preferably refers to a single nucleotide substitution. Typically, between different genomes or between different individuals, the polymorphic site may be occupied by two different nucleotides.

The terms "biallelic polymorphism" and "biallelic marker" are used interchangeably herein to refer to a polymorphism having two alleles at a fairly high frequency in the population, preferably a single nucleotide polymorphism. A "biallelic marker allele" refers to the nucleotide variants present at a biallelic marker site. Typically the frequency of the less common allele of the biallelic markers of the present invention has been validated to be greater than 1%, preferably the frequency is greater than 10%, more preferably the frequency is at least 20% (i.e. heterozygosity rate of at least 0.32), even more preferably the frequency is at least 30% (i.e. heterozygosity rate of at least 0.42). A biallelic marker wherein the frequency of the less common allele is 30% or more is termed a "high quality biallelic marker."

The location of nucleotides in a polynucleotide with respect to the center of the polynucleotide are described herein in the following manner. When a polynucleotide has an odd number of nucleotides, the nucleotide at an equal distance from the 3' and 5' ends of the polynucleotide is considered to be "at the center" of the polynucleotide, and any nucleotide immediately adjacent to the nucleotide at the center, or the nucleotide at the center itself is considered to be "within 1 nucleotide of the center." With an odd number of nucleotides in a polynucleotide any of the five nucleotides positions in the middle of the polynucleotide would be considered to be within 2 nucleotides of the center, and so on. When a polynucleotide has an even number of nucleotides, there would be a bond and not a nucleotide at the center of the polynucleotide. Thus, either of the two central nucleotides would be considered to be "within 1 nucleotide of the center" and any of the four nucleotides in the middle of the polynucleotide would be considered to be "within 2 nucleotides of the center", and so on. For polymorphisms which involve the substitution, insertion or deletion of 1 or more nucleotides, the polymorphism, allele or biallelic marker is "at the center" of a polynucleotide if the difference between the distance from the substituted, inserted, or deleted polynucleotides of the polymorphism and the 3' end of the polynucleotide, and the distance from the substituted,

inserted, or deleted polynucleotides of the polymorphism and the 5' end of the polynucleotide is zero or one nucleotide. If this difference is 0 to 3, then the polymorphism is considered to be "within 1 nucleotide of the center." If the difference is 0 to 5, the polymorphism is considered to be "within 2 nucleotides of the center." If the difference is 0 to 7, the polymorphism is considered to be "within 3 nucleotides of the center," and so on. For polymorphisms which involve the substitution, insertion or deletion of 1 or more nucleotides, the polymorphism, allele or biallelic marker is "at the center" of a polynucleotide if the difference between the distance from the substituted, inserted, or deleted polynucleotides of the polymorphism and the 3' end of the polynucleotide, and the distance from the substituted, inserted, or deleted polynucleotides of the polymorphism and the 5' end of the polynucleotide is zero or one nucleotide. If this difference is 0 to 3, then the polymorphism is considered to be "within 1 nucleotide of the center." If the difference is 0 to 5, the polymorphism is considered to be "within 2 nucleotides of the center." If the difference is 0 to 7, the polymorphism is considered to be "within 3 nucleotides of the center," and so on.

The term "upstream" is used herein to refer to a location which, is toward the 5' end of the polynucleotide from a specific reference point.

The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein to refer to nucleotides which can be hydrogen bonded to one another be virtue of their sequence identities in a manner like that found in double-helical DNA with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds (See Stryer, L., *Biochemistry,* 4th edition, 1995).

The terms "complementary" or "complement thereof" are used herein to refer to the sequences of polynucleotides which is capable of forming Watson & Crick base pairing with another specified polynucleotide throughout the entirety of the complementary region. This term is applied to pairs of polynucleotides based solely upon their sequences and not any particular set of conditions under which the two polynucleotides would actually bind.

As used herein the term "map-related biallelic marker" relates to a biallelic marker in linkage disequilibrium with any of the sequences disclosed in SEQ ID Nos. 1 to 3908 which contain a biallelic marker of the map. The term map-related biallelic marker encompasses all of the biallelic markers disclosed in SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908. The preferred map-related biallelic marker alleles of the present invention include each one of the alleles selected individually or in any combination from the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908, as identified in field <223> of the allele feature in the appended Sequence Listing, individually or in groups consisting of all the possible combinations of the alleles.

The terms "1ST allele" and "2ND allele" refer to the nucleotide located at the polymorphic base of a polynucleotide sequence containing a biallelic marker, as identified in field <222> of the allele feature in the appended Sequence Listing for each Sequence ID number. As used herein, the polymorphic base is located at nucleotide position 24 for each of SEQ ID Nos. 1 to 3908, with the exception of SEQ ID Nos. 914, 1013, 2544, 3434, 3795, and 3028. The polymorphic base is located at nucleotide position 23 for SEQ ID Nos. 914, 1013 and 2544, at nucleotide position 21 for SEQ ID No. 3028, at nucleotide position 20 for SEQ ID No. 3434.

I. Biallelic Markers And Polynucleotides Comprising Biallelic Markers

Polynucleotides of the present invention

The present invention encompasses polynucleotides for use as primers and probes in the methods of the invention. All of the polynucleotides of the invention may be specified as being isolated, purified or recombinant. These polynucleotides may consist of, consist essentially of, or comprise a contiguous span of nucleotides of a sequence from any sequence in the Sequence Listing as well as sequences which are complementary thereto ("complements thereof"). The "contiguous span" may be at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID. It should be noted that the polynucleotides of the present invention are not limited to having the exact flanking sequences surrounding the polymorphic bases which, are enumerated in the Sequence Listing. Rather, it will be appreciated that the flanking sequences surrounding the biallelic markers, or any of the primers of probes of the invention which, are more distant from the markers, may be lengthened or shortened to any extent compatible with their intended use and the present invention specifically contemplates such sequences. It will be appreciated that the polynucleotides referred to in the Sequence Listing may be of any length compatible with their intended use. Also the flanking regions outside of the contiguous span need not be homologous to native flanking sequences which actually occur in human subjects. The addition of any nucleotide sequence, which is compatible with the nucleotides intended use is specifically contemplated. The contiguous span may optionally include the map-related biallelic marker in said sequence. Biallelic markers generally consist of a polymorphism at one single base position. Each biallelic marker therefore corresponds to two forms of a polynucleotide sequence which, when compared with one another, present a nucleotide modification at one position. Usually, the nucleotide modification involves the substitution of one nucleotide for another. Optionally either the 1ST allele or the 2ND allele of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 may be specified as being present at the map-related biallelic marker.

Preferred polynucleotides may consist of, consist essentially of, or comprise a contiguous span of nucleotides of a sequence from SEQ ID Nos. 1 to 2260 as well as sequences which are complementary thereto. The "contiguous span" may be at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID. Particularly preferred are polynucleotides which consist of, consist essentially of, or comprise a contiguous span of nucleotides of a sequence of any of SEQ ID Nos. 1 to 2260, or the complements thereof, wherein the 1ST allele of the biallelic marker of the SEQ ID No. is present at the map-related biallelic marker. Other preferred polynucleotides consist of, consist essentially of, or comprise a contiguous span of nucleotides of any of SEQ ID Nos. 1 to 2260, or the complements thereof, wherein the 2ND allele of the biallelic marker of the SEQ ID No. is present at the map-related biallelic marker. Preferred polynucleotides may consist of, consist essentially of, or comprise a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID No., of a sequence from SEQ ID Nos. 2261 to 3734 as well as sequences which are complementary thereto. Particularly preferred are polynucleotides which consist of, consist essentially of, or comprise a contiguous span of nucleotides of a sequence of any of SEQ ID Nos. 2261 to 3734, or the complements thereof, wherein the 1ST allele of the biallelic marker of the SEQ ID No. is present at the map-related biallelic marker. Other preferred polynucleotides consist of, consist essentially of, or comprise a contiguous span of nucleotides of any of SEQ ID Nos. 2261 to 3734, or the complements thereof, wherein the 2ND allele of the biallelic marker of the SEQ ID No. is present at the map-related biallelic marker. Preferred polynucleotides may consist of, consist essentially of, or comprise a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID No., of a sequence from SEQ ID Nos. 3735 to 3908 as well as sequences which are complementary thereto. Particularly preferred are polynucleotides which consist of, consist essentially of, or comprise a contiguous span of nucleotides of a sequence of any of SEQ ID Nos. 3735 to 3908, or the complements thereof, wherein the 1ST allele of the biallelic marker of the SEQ ID No. is present at the map-related biallelic marker. Other preferred polynucleotides consist of, consist essentially of, or comprise a contiguous span of nucleotides of any of SEQ ID Nos. 3735 to 3908, or the complements thereof, wherein the 2ND allele of the biallelic marker of the SEQ ID No. is present at the map-related biallelic marker. Also encompassed by the polynucleotides of the present invention are polynucleotides which consist of, consist essentially of, or comprise a contiguous span at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45,

46 or 47 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of a sequence from SEQ ID Nos. 1201, 3242, 3907 and 3908 as well as sequences which are complementary thereto, wherein said contiguous span of SEQ ID Nos. 1201 or 3242 contains a "G" at the polymorphic base, or wherein said contiguous span of SEQ ID Nos. 3907 or 3908 contain an "A" at the polymorphic base.

The invention also relates to polynucleotides that hybridize, under conditions of high or intermediate stringency, to a polynucleotide of a sequence from any of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 as well as sequences which are complementary thereto. Preferably such polynucleotides are at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides in length, to the extent that a polynucleotide of these lengths is consistent with the lengths of the particular Sequence ID. Preferred polynucleotides comprise a map-related biallelic marker. Optionally either the 1^{ST} or the 2^{ND} allele of the biallelic markers disclosed in the SEQ ID No. may be specified as being present at the map-related biallelic marker. Conditions of high and intermediate stringency are further described in III.C.4.

The primers of the present invention may be designed from the disclosed sequences using any method known in the art. A preferred set of primers is fashioned such that the 3' end of the contiguous span of identity with the sequences of the Sequence Listing is present at the 3' end of the primer. Such a configuration allows the 3' end of the primer to hybridize to a selected nucleic acid sequence and dramatically increases the efficiency of the primer for amplification or sequencing reactions.

In a preferred set of primers the contiguous span is found in one of the sequences described in SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 or the complements thereof. The invention also relates to polynucleotides consisting of, consisting essentially of, or comprising a contiguous span of nucleotides of a sequence from SEQ ID Nos. 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773, as well as sequences which are complementary thereto, wherein the "contiguous span" may be at least 8, 10, 12, 15, 18, 19, 20, or 21 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID No.

Allele specific primers may be designed such that a biallelic marker is at the 3' end of the contiguous span and the contiguous span is present at the 3' end of the primer. Such allele specific primers tend to selectively prime an amplification or sequencing reaction so long as they are used with a nucleic acid sample that contains one of the two alleles present at a

biallelic marker. The 3' end of primer of the invention may be located within or at least 2, 4, 6, 8, 10, to the extent that this distance is consistent with the particular Sequence ID, nucleotides upstream of a map-related biallelic marker in said sequence or at any other location which is appropriate for their intended use in sequencing, amplification or the location of novel sequences or markers. Primers with their 3' ends located 1 nucleotide upstream of a map-related biallelic marker have a special utility as microsequencing assays. Preferred microsequencing primers are described in SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908, where for each of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908, the sense microsequencing primer contains the complement of the 19 nucleotides having their 3' ends located 1 nucleotide upstream of the polymorphic base of the respective SEQ ID No, and where the antisense microsequencing primer contains the complement of the 19 nucleotides of the complementary strand, nucleotides of the primer having their 3' end located 1 nucleotide upstream of the polymorphic base on the complementary strand to the respective SEQ ID No. The most preferred of said microsequencing primers for each of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 are microsequencing primers indicated as "A" or "S" in Table 1, which have been validated in microsequencing experiments.

The probes of the present invention may be designed from the disclosed sequences for any method known in the art, particularly methods which allow for testing if a particular sequence or marker disclosed herein is present. A preferred set of probes may be designed for use in the hybridization assays of the invention in any manner known in the art such that they selectively bind to one allele of a biallelic marker, but not the other under any particular set of assay conditions. Preferred hybridization probes may consists of, consist essentially of, or comprise a contiguous span of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908, or the complement thereof, which ranges in length from least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID No., or be specified as being 12, 15, 18, 19, 20, 25, 35, 40, 43, 44, 45, 46 or 47 nucleotides in length and including the map-related biallelic marker of said sequence. Optionally the 1st allele or 2nd allele of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 may be specified as being present at the biallelic marker site. Optionally, said biallelic marker may be within 6, 5, 4, 3, 2, or 1 nucleotides of the center of the hybridization probe or at the center of said probe.

Any of the polynucleotides of the present invention can be labeled, if desired, by incorporating a label detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive substances, fluorescent dyes or biotin. Preferably, polynucleotides are labeled at their 3' and

5' ends. A label can also be used to capture the primer, so as to facilitate the immobilization of either the primer or a primer extension product, such as amplified DNA, on a solid support. A capture label is attached to the primers or probes and can be a specific binding member which forms a binding pair with the solid's phase reagent's specific binding member (e.g. biotin and streptavidin). Therefore depending upon the type of label carried by a polynucleotide or a probe, it may be employed to capture or to detect the target DNA. Further, it will be understood that the polynucleotides, primers or probes provided herein, may, themselves, serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of a primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where a polynucleotide probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the target. In the case where a polynucleotide primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase. DNA Labeling techniques are well known to the skilled technician.

Any of the polynucleotides, primers and probes of the present invention can be conveniently immobilized on a solid support. Solid supports are known to those skilled in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells, duracytes® and others. The solid support is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and duracytes are all suitable examples. Suitable methods for immobilizing nucleic acids on solid phases include ionic, hydrophobic, covalent interactions and the like. A solid support, as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid support can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid support and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid support material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or

silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, duracytes® and other configurations known to those of ordinary skill in the art. The polynucleotides of the invention can be attached to or immobilized on a solid support individually or in groups of at least 2, 5, 8, 10, 12, 15, 20, or 25 distinct polynucleotides of the inventions to a single solid support. In addition, polynucleotides other than those of the invention may attached to the same solid support as one or more polynucleotides of the invention.

Any polynucleotide provided herein may be attached in overlapping areas or at random locations on the solid support. Alternatively the polynucleotides of the invention may be attached in an ordered array wherein each polynucleotide is attached to a distinct region of the solid support which does not overlap with the attachment site of any other polynucleotide. Preferably, such an ordered array of polynucleotides is designed to be "addressable" where the distinct locations are recorded and can be accessed as part of an assay procedure. Addressable polynucleotide arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. The knowledge of the precise location of each polynucleotides location makes these "addressable" arrays particularly useful in hybridization assays. Any addressable array technology known in the art can be employed with the polynucleotides of the invention. One particular embodiment of these polynucleotide arrays is known as the Genechips™, and has been generally described in US Patent 5,143,854; PCT publications WO 90/15070 and 92/10092. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods, which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis (Fodor et al., Science, 251:767-777, 1991). The immobilization of arrays of oligonucleotides on solid supports has been rendered possible by the development of a technology generally identified as "Very Large Scale Immobilized Polymer Synthesis" (VLSIPS™) in which, typically, probes are immobilized in a high density array on a solid surface of a chip. examples of VLSIPS™ technologies are provided in US Patents 5,143,854 and 5,412,087 and in PCT Publications WO 90/15070, WO 92/10092 and WO 95/11995, which describe methods for forming oligonucleotide arrays through techniques such as light-directed synthesis techniques. In designing strategies aimed at providing arrays of nucleotides immobilized on solid supports, further presentation strategies were developed to order and display the oligonucleotide arrays on the chips in an attempt to maximize hybridization patterns and sequence information. examples of such presentation strategies are disclosed in PCT Publications WO 94/12305, WO 94/11530, WO 97/29212 and WO 97/31256.

Oligonucleotide arrays may comprise at least one of the sequences selected

from the group consisting of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 and the sequences complementary thereto, or a fragment thereof of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 consecutive nucleotides, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for determining whether a sample contains one or more alleles of the biallelic markers of the present invention. Oligonucleotide arrays may also comprise at least one of the sequences selected from the group consisting of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908, and the sequences complementary thereto, or a fragment thereof of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 consecutive nucleotides, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for amplifying one or more alleles of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908. In other embodiments, arrays may also comprise at least one of the sequences selected from the group consisting of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 and the sequences complementary thereto, or a fragment thereof of at 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 consecutive nucleotides, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for conducting microsequencing analyses to determine whether a sample contains one or more alleles of the biallelic markers of the invention. In still further embodiments, the oligonucleotide array may comprise at least one of the sequences selecting from the group consisting of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 and the sequences complementary thereto, or a fragment thereof of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides in length, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for determining whether a sample contains one or more alleles of the biallelic markers of the present invention.

In designing strategies aimed at providing arrays of nucleotides immobilized on solid supports, further presentation strategies were developed to order and display the probe arrays on the chips in an attempt to maximize hybridization patterns and sequence information. Examples of such presentation strategies are disclosed in PCT Publications WO 94/12305, WO 94/11530, WO 97/29212 and WO 97/31256.

Each DNA chip can contain thousands to millions of individual synthetic DNA probes arranged in a grid-like pattern and miniaturized to the size of a dime. In some embodiments, the efficiency of hybridization of nucleic acids in the sample with the probes attached to the chip may be improved by using polyacrylamide gel pads isolated from one another by hydrophobic regions in which the DNA probes are covalently linked to an acrylamide matrix.

The polymorphic bases present in the biallelic marker or markers of the sample nucleic acids are determined as follows. Probes which contain at least a portion of one or more of the biallelic markers of the present invention are synthesized either *in situ* or by conventional synthesis and immobilized on an appropriate chip using methods known to the skilled technician.

Any one or more alleles of the biallelic markers described herein (SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto) or fragments thereof containing the polymorphic bases, may be fixed to a solid support, such as a microchip or other immobilizing surface. The fragments of these nucleic acids may comprise at least 10, at least 15, at least 20, at least 25, or more than 25 consecutive nucleotides of the biallelic markers described herein. Preferably, the fragments include the polymorphic bases of the biallelic markers.

A nucleic acid sample is applied to the immobilizing surface and analyzed to determine the identities of the polymorphic bases of one or more of the biallelic markers. In some embodiments, the solid support may also include one or more of the amplification primers described herein, or fragments comprising at least 10, at least 15, or at least 20 consecutive nucleotides thereof, for generating an amplification product containing the polymorphic bases of the biallelic markers to be analyzed in the sample.

Another embodiment of the present invention is a solid support which includes one or more of the microsequencing primers of the invention, or fragments comprising at least 10, at least 15, or at least 20 consecutive nucleotides thereof and having a 3' terminus immediately upstream of the polymorphic base of the corresponding biallelic marker, for determining the identity of the polymorphic base of the one or more biallelic markers fixed to the solid support.

For example, one embodiment of the present invention is an array of nucleic acids fixed to a solid support, such as a microchip, bead, or other immobilizing surface, comprising one or more of the biallelic markers in the maps of the present invention or a fragment comprising at least 10, at least 15, at least 20, at least 25, or more than 25 consecutive nucleotides thereof including the polymorphic base. For example, the array may comprise 1, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, or 3000 of the biallelic markers selected from the group consisting of SEQ ID Nos.: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto, or a fragment comprising at least 10, at least 15, at least 20, at least 25,

or more than 25 consecutive nucleotides thereof including the polymorphic base.

Another embodiment of the present invention is an array comprising amplification primers for generating amplification products containing the polymorphic bases of one or more, at least five, at least 10, at least 20, at least 100, at least 200, at least 300, at least 400, or more than 400 of the biallelic markers in the maps of the present invention. For example, the array may comprise amplification primers for generating amplification products containing the polymorphic bases of at least 1,5, 10, 20, 50, 100, 200, 300, 400, 500, 1000, 2000, or 3000, of the biallelic markers selected from the group consisting of SEQ ID Nos.: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto. In such arrays, the amplification primers included in the array are capable of amplifying the biallelic marker sequences to be detected in the nucleic acid sample applied to the array (i.e. the amplification primers correspond to the biallelic markers affixed to the array - see Table 1). Thus, the arrays may include one or more of the amplification primers of SEQ ID Nos.: 3935 to 7842, 7866 to 11773, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 10125, 10126 to 11599, and 11600 to 11773 corresponding to the one or more biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 which are included in the array.

Another embodiment of the present invention is an array which includes microsequencing primers capable of determining the identity of the polymorphic bases of at least 1, 5, 10, 20, 50, 100, 200, 300, 500, 1000, 2000, or 3000 of the present invention. For example, the array may comprise microsequencing primers capable of determining the identity of the polymorphic bases of one or more, at least five, at least 10, at least 20, at least 100, at least 200, at least 300, at least 400, or more than 400 of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto.

Arrays containing any combination of the above nucleic acids which permits the specific detection or identification of the polymorphic bases of the biallelic markers in the maps of the present invention, including any combination of biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto are also within the scope of the present invention. For example, the array may comprise both the biallelic markers and amplification primers capable of generating amplification products containing the polymorphic bases of the biallelic markers. Alternatively, the array may comprise both amplification primers capable of generating amplification products containing the polymorphic bases of the biallelic markers and microsequencing primers capable of determining the identities of the polymorphic bases of these markers.

Although the above examples describe arrays comprising specific groups of biallelic markers and, in some embodiments, specific amplification primers and microsequencing primers, it will be appreciated that the present invention encompasses arrays including any

biallelic marker, group of biallelic markers, amplification primer, group of amplification primers, microsequencing primer, or group of amplification primers described herein, as well as any combination of the preceding nucleic acids.

The present invention also encompasses diagnostic kits comprising one or more polynucleotides of the invention, optionally with a portion or all of the necessary reagents and instructions for genotyping a test subject by determining the identity of a nucleotide at a map-related biallelic marker. The polynucleotides of a kit may optionally be attached to a solid support, or be part of an array or addressable array of polynucleotides. The kit may provide for the determination of the identity of the nucleotide at a marker position by any method known in the art including, but not limited to, a sequencing assay method, a microsequencing assay method, a hybridization assay method, or an allele specific amplification method. Optionally such a kit may include instructions for scoring the results of the determination with respect to the test subjects' risk of contracting a diseases involving a disease, likely response to an agent acting on a disease, or chances of suffering from side effects to an agent acting on a disease.

II. Methods For *De Novo* Identification Of Biallelic Markers

Any of a variety of methods can be used to screen a genomic fragment for single nucleotide polymorphisms such as differential hybridization with oligonucleotide probes, detection of changes in the mobility measured by gel electrophoresis or direct sequencing of the amplified nucleic acid. A preferred method for identifying biallelic markers involves comparative sequencing of genomic DNA fragments from an appropriate number of unrelated individuals.

In a first embodiment, DNA samples from unrelated individuals are pooled together, following which the genomic DNA of interest is amplified and sequenced. The nucleotide sequences thus obtained are then analyzed to identify significant polymorphisms. One of the major advantages of this method resides in the fact that the pooling of the DNA samples substantially reduces the number of DNA amplification reactions and sequencing reactions, which must be carried out. Moreover, this method is sufficiently sensitive so that a biallelic marker obtained thereby usually demonstrates a sufficient frequency of its less common allele to be useful in conducting association studies. Usually, the frequency of the least common allele of a biallelic marker identified by this method is at least 10%.

In a second embodiment, the DNA samples are not pooled and are therefore amplified and sequenced individually. This method is usually preferred when biallelic markers need to be identified in order to perform association studies within candidate genes. Preferably, highly relevant gene regions such as promoter regions or exon regions may be screened for biallelic markers. A biallelic marker obtained using this method may show a lower degree of

informativeness for conducting association studies, e.g. if the frequency of its less frequent allele may be less than about 10%. Such a biallelic marker will however be sufficiently informative to conduct association studies and it will further be appreciated that including less informative biallelic markers in the genetic analysis studies of the present invention, may allow in some cases the direct identification of causal mutations, which may, depending on their penetrance, be rare mutations.

The following is a description of the various parameters of a preferred method used by the inventors for the identification of the biallelic markers of the present invention.

II.A. Genomic DNA samples

The genomic DNA samples from which the biallelic markers of the present invention are generated are preferably obtained from unrelated individuals corresponding to a heterogeneous population of known ethnic background. The number of individuals from whom DNA samples are obtained can vary substantially, preferably from about 10 to about 1000, more preferably from about 50 to about 200 individuals. Usually, DNA samples are collected from at least about 100 individuals in order to have sufficient polymorphic diversity in a given population to identify as many markers as possible and to generate statistically significant results.

As for the source of the genomic DNA to be subjected to analysis, any test sample can be foreseen without any particular limitation. These test samples include biological samples, which can be tested by the methods of the present invention described herein, and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk, white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; fixed tissue specimens including tumor and non-tumor tissue and lymph node tissues; bone marrow aspirates and fixed cell specimens. The preferred source of genomic DNA used in the present invention is from peripheral venous blood of each donor. Techniques to prepare genomic DNA from biological samples are well known to the skilled technician. Details of a preferred embodiment are provided in Example 27. The person skilled in the art can choose to amplify pooled or unpooled DNA samples.

II.B. DNA Amplification

The identification of biallelic markers in a sample of genomic DNA may be facilitated through the use of DNA amplification methods. DNA samples can be pooled or unpooled for the amplification step. DNA amplification techniques are well known to those skilled in the art. Various methods to amplify DNA fragments carrying biallelic markers are further described hereinafter in III.B. The PCR technology is the preferred amplification technique used to identify new biallelic markers.

In a first embodiment, biallelic markers are identified using genomic sequence information generated by the inventors. Genomic DNA fragments, such as the inserts of the BAC clones described above, are sequenced and used to design primers for the amplification of 500 bp fragments. These 500 bp fragments are amplified from genomic DNA and are scanned for biallelic markers. Primers may be designed using the OSP software (Hillier L. and Green P., 1991). All primers may contain, upstream of the specific target bases, a common oligonucleotide tail that serves as a sequencing primer. Those skilled in the art are familiar with primer extensions, which can be used for these purposes.

In another embodiment of the invention, genomic sequences of candidate genes are available in public databases allowing direct screening for biallelic markers. Preferred primers, useful for the amplification of genomic sequences encoding the candidate genes, focus on promoters, exons and splice sites of the genes. A biallelic marker present in these functional regions of the gene have a higher probability to be a causal mutation.

Preferred primers include those disclosed in SEQ ID Nos. 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773.

II.C. Sequencing Of Amplified Genomic DNA And Identification Of Single Nucleotide Polymorphisms

The amplification products generated as described above, are then sequenced using any method known and available to the skilled technician. Methods for sequencing DNA using either the dideoxy-mediated method (Sanger method) or the Maxam-Gilbert method are widely known to those of ordinary skill in the art. Such methods are for example disclosed in Maniatis et al. (Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Second Edition, 1989). Alternative approaches include hybridization to high-density DNA probe arrays as described in Chee et al. (Science 274, 610, 1996).

Preferably, the amplified DNA is subjected to automated dideoxy terminator sequencing reactions using a dye-primer cycle sequencing protocol. The products of the sequencing reactions are run on sequencing gels and the sequences are determined using gel image analysis. The polymorphism search is based on the presence of superimposed peaks in the electrophoresis pattern resulting from different bases occurring at the same position. Because each dideoxy terminator is labeled with a different fluorescent molecule, the two peaks corresponding to a biallelic site present distinct colors corresponding to two different nucleotides at the same position on the sequence. However, the presence of two peaks can be an artifact due to background noise. To exclude such an artifact, the two DNA strands are sequenced and a comparison between the peaks is carried out. In order to be registered as a polymorphic sequence, the polymorphism has to be detected on both strands.

The above procedure permits those amplification products, which contain biallelic markers to be identified. The detection limit for the frequency of biallelic polymorphisms detected by sequencing pools of 100 individuals is approximately 0.1 for the minor allele, as verified by sequencing pools of known allelic frequencies. However, more than 90% of the biallelic polymorphisms detected by the pooling method have a frequency for the minor allele higher than 0.25. Therefore, the biallelic markers selected by this method have a frequency of at least 0.1 for the minor allele and less than 0.9 for the major allele. Preferably at least 0.2 for the minor allele and less than 0.8 for the major allele, more preferably at least 0.3 for the minor allele and less than 0.7 for the major allele, thus a heterozygosity rate higher than 0.18, preferably higher than 0.32, more preferably higher than 0.42.

In another embodiment, biallelic markers are detected by sequencing individual DNA samples, the frequency of the minor allele of such a biallelic marker may be less than 0.1.

The markers carried by the same fragment of genomic DNA, such as the insert in a BAC clone, need not necessarily be ordered with respect to one another within the genomic fragment to conduct association studies. However, in some embodiments of the present invention, the order of biallelic markers carried by the same fragment of genomic DNA are determined.

II.D. Validation of the biallelic markers of the present invention

The polymorphisms are evaluated for their usefulness as genetic markers by validating that both alleles are present in a population. Validation of the biallelic markers is accomplished by genotyping a group of individuals by a method of the invention and demonstrating that both alleles are present. Microsequencing is a preferred method of genotyping alleles. The validation by genotyping step may be performed on individual samples derived from each individual in the group or by genotyping a pooled sample derived from more than one individual. The group can be as small as one individual if that individual is heterozygous for the allele in question. Preferably the group contains at least three individuals, more preferably the group contains five or six individuals, so that a single validation test will be more likely to result in the validation of more of the biallelic markers that are being tested. It should be noted, however, that when the validation test is performed on a small group it may result in a false negative result if as a result of sampling error none of the individuals tested carries one of the two alleles. Thus, the validation process is less useful in demonstrating that a particular initial result is an artifact, than it is at demonstrating that there is a *bona fide* biallelic marker at a particular position in a sequence. All of the genotyping, haplotyping, association, and interaction study methods of the invention may optionally be performed solely with validated biallelic markers.

II.E. Evaluati n of the frequency of the biallelic markers of the present invention

The validated biallelic markers are further evaluated for their usefulness as genetic markers by determining the frequency of the least common allele at the biallelic marker site. The determination of the least common allele is accomplished by genotyping a group of individuals by a method of the invention and demonstrating that both alleles are present. This determination of frequency by genotyping step may be performed on individual samples derived from each individual in the group or by genotyping a pooled sample derived from more than one individual. The group must be large enough to be representative of the population as a whole. Preferably the group contains at least 20 individuals, more preferably the group contains at least 50 individuals, most preferably the group contains at least 100 individuals. Of course the larger the group the greater the accuracy of the frequency determination because of reduced sampling error. A biallelic marker wherein the frequency of the less common allele is 30% or more is termed a "high quality biallelic marker." All of the genotyping, haplotyping, association, and interaction study methods of the invention may optionally be performed solely with high quality biallelic markers.

III. Methods Of Genotyping An Individual For Biallelic Markers

Methods are provided to genotype a biological sample for one or more biallelic markers of the present invention, all of which may be performed *in vitro*. Such methods of genotyping comprise determining the identity of a nucleotide at a map-related biallelic marker by any method known in the art. These methods find use in genotyping case-control populations in association studies as well as individuals in the context of detection of alleles of biallelic markers which, are known to be associated with a given trait, in which case both copies of the biallelic marker present in individual's genome are determined so that an individual may be classified as homozygous or heterozygous for a particular allele.

These genotyping methods can be performed nucleic acid samples derived from a single individual or pooled DNA samples.

Genotyping can be performed using similar methods as those described above for the identification of the biallelic markers, or using other genotyping methods such as those further described below. In preferred embodiments, the comparison of sequences of amplified genomic fragments from different individuals is used to identify new biallelic markers whereas microsequencing is used for genotyping known biallelic markers in diagnostic and association study applications.

III.A. Source of DNA for genotyping

Any source of nucleic acids, in purified or non-purified form, can be utilized as the starting nucleic acid, provided it contains or is suspected of containing the specific nucleic acid sequence desired. DNA or RNA may be extracted from cells, tissues, body fluids and the like as described above in II.A. While nucleic acids for use in the genotyping methods of the

invention can be derived from any mammalian source, the test subjects and individuals from which nucleic acid samples are taken are generally understood to be human.

III.B. Amplification Of DNA Fragments Comprising Biallelic Markers

Methods and polynucleotides are provided to amplify a segment of nucleotides comprising one or more biallelic marker of the present invention. It will be appreciated that amplification of DNA fragments comprising biallelic markers may be used in various methods and for various purposes and is not restricted to genotyping. Nevertheless, many genotyping methods, although not all, require the previous amplification of the DNA region carrying the biallelic marker of interest. Such methods specifically increase the concentration or total number of sequences that span the biallelic marker or include that site and sequences located either distal or proximal to it. Diagnostic assays may also rely on amplification of DNA segments carrying a biallelic marker of the present invention.

Amplification of DNA may be achieved by any method known in the art. The established PCR (polymerase chain reaction) method or by developments thereof or alternatives. Amplification methods which can be utilized herein include but are not limited to Ligase Chain Reaction (LCR) as described in EP A 320 308 and EP A 439 182, Gap LCR (Wolcott, M.J., Clin. Mcrobiol. Rev. 5:370-386), the so-called "NASBA" or "3SR" technique described in Guatelli J.C. et al. (*Proc. Natl. Acad. Sci. USA* 87:1874-1878, 1990) and in Compton J. (*Nature* 350:91-92, 1991), Q-beta amplification as described in European Patent Application no 4544610, strand displacement amplification as described in Walker et al. (*Clin. Chem.* 42:9-13, 1996) and EP A 684 315 and, target mediated amplification as described in PCT Publication WO 9322461.

LCR and Gap LCR are exponential amplification techniques, both depend on DNA ligase to join adjacent primers annealed to a DNA molecule. In Ligase Chain Reaction (LCR), probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess to target. The first probe hybridizes to a first segment of the target strand and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3'hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated

products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. A method for multiplex LCR has also been described (WO 9320227). Gap LCR (GLCR) is a version of LCR where the probes are not adjacent but are separated by 2 to 3 bases.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770 or, to use Asymmetric Gap LCR (RT-AGLCR) as described by Marshall R.L. et al. (*PCR Methods and Applications* 4:80-84, 1994). AGLCR is a modification of GLCR that allows the amplification of RNA.

Some of these amplification methods are particularly suited for the detection of single nucleotide polymorphisms and allow the simultaneous amplification of a target sequence and the identification of the polymorphic nucleotide as it is further described in III.C.

The PCR technology is the preferred amplification technique used in the present invention. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see Molecular Cloning to Genetic Engineering White, B.A. Ed. in *Methods in Molecular Biology* 67: Humana Press, Totowa (1997) and the publication entitled "PCR Methods and Applications" (1991, Cold Spring Harbor Laboratory Press). In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites. PCR has further been described in several patents including US Patents 4,683,195, 4,683,202 and 4,965,188.

The identification of biallelic markers as described above allows the design of appropriate oligonucleotides, which can be used as primers to amplify DNA fragments comprising the biallelic markers of the present invention. Amplification can be performed using the primers initially used to discover new biallelic markers which are described herein or any set of primers allowing the amplification of a DNA fragment comprising a biallelic marker of the present invention. Primers can be prepared by any suitable method. As for example, direct chemical synthesis by a method such as the phosphodiester method of Narang S.A. et al. (*Methods Enzymol.* 68:90-98, 1979), the phosphodiester method of Brown E.L. et al. (*Methods Enzymol.* 68:109-151, 1979), the diethylphosphoramidite method of Beaucage et al. (*Tetrahedron Lett.* 22:1859-1862, 1981) and the solid support method described in EP 0

707 592.

In some embodiments the present invention provides primers for amplifying a DNA fragment containing one or more biallelic markers of the present invention. Preferred amplification primers are listed in SEQ ID Nos. 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. It will be appreciated that the primers listed are merely exemplary and that any other set of primers which produce amplification products containing one or more biallelic markers of the present invention.

The primers are selected to be substantially complementary to the different strands of each specific sequence to be amplified. The length of the primers of the present invention can range from 8 to 100 nucleotides, preferably from 8 to 50, 8 to 30 or more preferably 8 to 25 nucleotides. Shorter primers tend to lack specificity for a target nucleic acid sequence and generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. Longer primers are expensive to produce and can sometimes self-hybridize to form hairpin structures. The formation of stable hybrids depends on the melting temperature (Tm) of the DNA. The Tm depends on the length of the primer, the ionic strength of the solution and the G+C content. The higher the G+C content of the primer, the higher is the melting temperature because G:C pairs are held by three H bonds whereas A:T pairs have only two. The G+C content of the amplification primers of the present invention preferably ranges between 10 and 75%, more preferably between 35 and 60%, and most preferably between 40 and 55%. The appropriate length for primers under a particular set of assay conditions may be empirically determined by one of skill in the art.

The spacing of the primers determines the length of the segment to be amplified. In the context of the present invention amplified segments carrying biallelic markers can range in size from at least about 25 bp to 35 kbp. Amplification fragments from 25-3000 bp are typical, fragments from 50-1000 bp are preferred and fragments from 100-600 bp are highly preferred. It will be appreciated that amplification primers for the biallelic markers may be any sequence which allow the specific amplification of any DNA fragment carrying the markers. Amplification primers may be labeled or immobilized on a solid support as described in I.

III.C. Methods of Genotyping DNA samples for Biallelic Markers

Any method known in the art can be used to identify the nucleotide present at a biallelic marker site. Since the biallelic marker allele to be detected has been identified and specified in the present invention, detection will prove simple for one of ordinary skill in the art by employing any of a number of techniques. Many genotyping methods require the previous amplification of the DNA region carrying the biallelic marker of interest. While the

amplification of target or signal is often preferred at present, ultrasensitive detection methods which do not require amplification are also encompassed by the present genotyping methods. Methods well-known to those skilled in the art that can be used to detect biallelic polymorphisms include methods such as, conventional dot blot analyzes, single strand conformational polymorphism analysis (SSCP) described by Orita et al. (*Proc. Natl. Acad. Sci. U.S.A* 86:27776-2770, 1989), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis, mismatch cleavage detection, and other conventional techniques as described in Sheffield, V.C. et al. (*Proc. Natl. Acad. Sci. USA* 49:699-706, 1991), White et al. (*Genomics* 12:301-306, 1992), Grompe, M. et al. (*Proc. Natl. Acad. Sci. USA* 86:5855-5892, 1989) and Grompe, M. (*Nature Genetics* 5:111-117, 1993). Another method for determining the identity of the nucleotide present at a particular polymorphic site employs a specialized exonuclease-resistant nucleotide derivative as described in US patent 4,656,127.

Preferred methods involve directly determining the identity of the nucleotide present at a biallelic marker site by sequencing assay, enzyme-based mismatch detection assay, or hybridization assay. The following is a description of some preferred methods. A highly preferred method is the microsequencing technique. The term "sequencing assay" is used herein to refer to polymerase extension of duplex primer/template complexes and includes both traditional sequencing and microsequencing.

1) Sequencing assays

The nucleotide present at a polymorphic site can be determined by sequencing methods. In a preferred embodiment, DNA samples are subjected to PCR amplification before sequencing as described above. DNA sequencing methods are described in IIC.

Preferably, the amplified DNA is subjected to automated dideoxy terminator sequencing reactions using a dye-primer cycle sequencing protocol. Sequence analysis allows the identification of the base present at the biallelic marker site.

2) Microsequencing assays

In microsequencing methods, a nucleotide at the polymorphic site that is unique to one of the alleles in a target DNA is detected by a single nucleotide primer extension reaction. This method involves appropriate microsequencing primers which, hybridize just upstream of a polymorphic base of interest in the target nucleic acid. A polymerase is used to specifically extend the 3' end of the primer with one single ddNTP (chain terminator) complementary to the selected nucleotide at the polymorphic site. Next the identity of the incorporated nucleotide is determined in any suitable way.

Typically, microsequencing reactions are carried out using fluorescent ddNTPs and the extended microsequencing primers are analyzed by electrophoresis on ABI 377 sequencing machines to determine the identity of the incorporated nucleotide as described in EP 412 883.

Alternatively capillary electrophoresis can be used in order to process a higher number of assays simultaneously. An example of a typical microsequencing procedure that can be used in the context of the present invention is provided in Example 8.

Different approaches can be used to detect the nucleotide added to the microsequencing primer. A homogeneous phase detection method based on fluorescence resonance energy transfer has been described by Chen and Kwok (*Nucleic Acids Research* 25:347-353 1997) and Chen et al. (*Proc. Natl. Acad. Sci. USA* 94/20 10756-10761,1997). In this method amplified genomic DNA fragments containing polymorphic sites are incubated with a 5'-fluorescein–labeled primer in the presence of allelic dye-labeled dideoxyribonucleoside triphosphates and a modified Taq polymerase. The dye-labeled primer is extended one base by the dye-terminator specific for the allele present on the template. At the end of the genotyping reaction, the fluorescence intensities of the two dyes in the reaction mixture are analyzed directly without separation or purification. All these steps can be performed in the same tube and the fluorescence changes can be monitored in real time. Alternatively, the extended primer may be analyzed by MALDI-TOF Mass Spectrometry. The base at the polymorphic site is identified by the mass added onto the microsequencing primer (see Haff L.A. and Smirnov I.P., *Genome Research*, 7:378-388, 1997).

Microsequencing may be achieved by the established microsequencing method or by developments or derivatives thereof. Alternative methods include several solid-phase microsequencing techniques. The basic microsequencing protocol is the same as described previously, except that the method is conducted as a heterogenous phase assay, in which the primer or the target molecule is immobilized or captured onto a solid support. To simplify the primer separation and the terminal nucleotide addition analysis, oligonucleotides are attached to solid supports or are modified in such ways that permit affinity separation as well as polymerase extension. The 5' ends and internal nucleotides of synthetic oligonucleotides can be modified in a number of different ways to permit different affinity separation approaches, e.g., biotinylation. If a single affinity group is used on the oligonucleotides, the oligonucleotides can be separated from the incorporated terminator regent. This eliminates the need of physical or size separation. More than one oligonucleotide can be separated from the terminator reagent and analyzed simultaneously if more than one affinity group is used. This permits the analysis of several nucleic acid species or more nucleic acid sequence information per extension reaction. The affinity group need not be on the priming oligonucleotide but could alternatively be present on the template. For example, immobilization can be carried out via an interaction between biotinylated DNA and streptavidin-coated microtitration wells or avidin-coated polystyrene particles. In the same manner oligonucleotides or templates may be attached to a solid support in a high-density

format. In such solid phase microsequencing reactions, incorporated ddNTPs can be radiolabeled (Syvänen, *Clinica Chimica Acta* 226:225-236, 1994) or linked to fluorescein (Livak and Hainer, *Human Mutation* 3:379-385,1994). The detection of radiolabeled ddNTPs can be achieved through scintillation-based techniques. The detection of fluorescein-linked ddNTPs can be based on the binding of antifluorescein antibody conjugated with alkaline phosphatase, followed by incubation with a chromogenic substrate (such as *p*-nitrophenyl phosphate). Other possible reporter-detection pairs include: ddNTP linked to dinitrophenyl (DNP) and anti-DNP alkaline phosphatase conjugate (Harju et al., *Clin. Chem.* 39/11 2282-2287, 1993) or biotinylated ddNTP and horseradish peroxidase-conjugated streptavidin with *o*-phenylenediamine as a substrate (WO 92/15712). As yet another alternative solid-phase microsequencing procedure, Nyren et al. (*Analytical Biochemistry* 208:171-175, 1993) described a method relying on the detection of DNA polymerase activity by an enzymatic luminometric inorganic pyrophosphate detection assay (ELIDA).

Pastinen et al. (*Genome research* 7:606-614, 1997) describe a method for multiplex detection of single nucleotide polymorphism in which the solid phase minisequencing principle is applied to an oligonucleotide array format. High-density arrays of DNA probes attached to a solid support (DNA chips) are further described in III.C.5.

In one aspect the present invention provides polynucleotides and methods to genotype one or more biallelic markers of the present invention by performing a microsequencing assay. In the preferred embodiment, it will be appreciated that any primer having a 3' end immediately adjacent to a polymorphic nucleotide may be used as a microsequencing primer. Similarly, it will be appreciated that microsequencing analysis may be performed for any biallelic marker or any combination of biallelic markers of the present invention. One aspect of the present invention is a solid support which includes one or more microsequencing primers comprising nucleotides complementary to the nucleotide sequences of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 or the complements thereof, or fragments comprising at least 8, at least 12, at least 15, or at least 20 consecutive nucleotides thereof and having a 3' terminus immediately upstream of the corresponding biallelic marker, for determining the identity of a nucleotide at biallelic marker site.

3) Mismatch detection assays based on polymerases and ligases

In one aspect the present invention provides polynucleotides and methods to determine the allele of one or more biallelic markers of the present invention in a biological sample, by mismatch detection assays based on polymerases and/or ligases. These assays are based on the specificity of polymerases and ligases. Polymerization reactions places particularly stringent requirements on correct base pairing of the 3' end of the amplification primer and the joining of two oligonucleotides hybridized to a target DNA sequence is quite

sensitive to mismatches close to the ligation site, especially at the 3' end. The terms "enzyme based mismatch detection assay" are used herein to refer to any method of determining the allele of a biallelic marker based on the specificity of ligases and polymerases. Preferred methods are described below. Methods, primers and various parameters to amplify DNA fragments comprising biallelic markers of the present invention are further described above in III.B.

Allele specific amplification

Discrimination between the two alleles of a biallelic marker can also be achieved by allele specific amplification, a selective strategy, whereby one of the alleles is amplified without amplification of the other allele. This is accomplished by placing a polymorphic base at the 3' end of one of the amplification primers. Because the extension forms from the 3'end of the primer, a mismatch at or near this position has an inhibitory effect on amplification. Therefore, under appropriate amplification conditions, these primers only direct amplification on their complementary allele. Designing the appropriate allele-specific primer and the corresponding assay conditions are well with the ordinary skill in the art.

Ligation/amplification based methods

The "Oligonucleotide Ligation Assay" (OLA) uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target molecules. One of the oligonucleotides is biotinylated, and the other is detectably labeled. If the precise complementary sequence is found in a target molecule, the oligonucleotides will hybridize such that their termini abut, and create a ligation substrate that can be captured and detected. OLA is capable of detecting biallelic markers and may be advantageously combined with PCR as described by Nickerson D.A. et al. (*Proc. Natl. Acad. Sci. U.S.A.* 87:8923-8927, 1990). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA.

Other methods which are particularly suited for the detection of biallelic markers include LCR (ligase chain reaction), Gap LCR (GLCR) which are described above in III.B. As mentioned above LCR uses two pairs of probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides, is selected to permit the pair to hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependant ligase. In accordance with the present invention, LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a biallelic marker site. In one embodiment, either oligonucleotide will be designed to include the biallelic marker site. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains or lacks the specific nucleotide(s) that is complementary to the biallelic marker on the oligonucleotide.

In an alternative embodiment, the oligonucleotides will not include the biallelic marker, such that when they hybridize to the target molecule, a "gap" is created as described in WO 90/01069. This gap is then "filled" with complementary dNTPs (as mediated by DNA polymerase), or by an additional pair of oligonucleotides. Thus at the end of each cycle, each single strand has a complement capable of serving as a target during the next cycle and exponential allele-specific amplification of the desired sequence is obtained.

Ligase/Polymerase-mediated Genetic Bit Analysis™ is another method for determining the identity of a nucleotide at a preselected site in a nucleic acid molecule (WO 95/21271). This method involves the incorporation of a nucleoside triphosphate that is complementary to the nucleotide present at the preselected site onto the terminus of a primer molecule, and their subsequent ligation to a second oligonucleotide. The reaction is monitored by detecting a specific label attached to the reaction's solid phase or by detection in solution.

4) Hybridization assay methods

A preferred method of determining the identity of the nucleotide present at a biallelic marker site involves nucleic acid hybridization. The hybridization probes, which can be conveniently used in such reactions, preferably include the probes defined herein. Any hybridization assay may be used including Southern hybridization, Northern hybridization, dot blot hybridization and solid-phase hybridization (see Sambrook et al., Molecular Cloning – A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., 1989).

Hybridization refers to the formation of a duplex structure by two single stranded nucleic acids due to complementary base pairing. Hybridization can occur between exactly complementary nucleic acid strands or between nucleic acid strands that contain minor regions of mismatch. Specific probes can be designed that hybridize to one form of a biallelic marker and not to the other and therefore are able to discriminate between different allelic forms. Allele-specific probes are often used in pairs, one member of a pair showing perfect match to a target sequence containing the original allele and the other showing a perfect match to the target sequence containing the alternative allele. Hybridization conditions should be sufficiently stringent that there is a significant difference in hybridization intensity between alleles, and preferably an essentially binary response, whereby a probe hybridizes to only one of the alleles. Stringent, sequence specific hybridization conditions, under which a probe will hybridize only to the exactly complementary target sequence are well known in the art (Sambrook et al., Molecular Cloning – A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., 1989). Stringent conditions are sequence dependent and will be different in different circumstances. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. By way of example and not limitation, procedures using conditions of high stringency are

as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C, the preferred hybridization temperature, in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Alternatively, the hybridization step can be performed at 65°C in the presence of SSC buffer, 1 x SSC corresponding to 0.15M NaCl and 0.05 M Na citrate. Subsequently, filter washes can be done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA, followed by a wash in 0.1X SSC at 50°C for 45 min. Alternatively, filter washes can be performed in a solution containing 2 x SSC and 0.1% SDS, or 0.5 x SSC and 0.1% SDS, or 0.1 x SSC and 0.1% SDS at 68°C for 15 minute intervals. Following the wash steps, the hybridized probes are detectable by autoradiography. By way of example and not limitation, procedures using conditions of intermediate stringency are as follows: Filters containing DNA are prehybridized, and then hybridized at a temperature of 60°C in the presence of a 5 x SSC buffer and labeled probe. Subsequently, filters washes are performed in a solution containing 2x SSC at 50°C and the hybridized probes are detectable by autoradiography. Other conditions of high and intermediate stringency which may be used are well known in the art and as cited in Sambrook et al. (Molecular Cloning - A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., 1989) and Ausubel et al. (Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y., 1989).

Although such hybridizations can be performed in solution, it is preferred to employ a solid-phase hybridization assay. The target DNA comprising a biallelic marker of the present invention may be amplified prior to the hybridization reaction. The presence of a specific allele in the sample is determined by detecting the presence or the absence of stable hybrid duplexes formed between the probe and the target DNA. The detection of hybrid duplexes can be carried out by a number of methods. Various detection assay formats are well known which utilize detectable labels bound to either the target or the probe to enable detection of the hybrid duplexes. Typically, hybridization duplexes are separated from unhybridized nucleic acids and the labels bound to the duplexes are then detected. Those skilled in the art will recognize that wash steps may be employed to wash away excess target DNA or probe. Standard heterogeneous assay formats are suitable for detecting the hybrids using the labels present on the primers and probes.

Two recently developed assays allow hybridization-based allele discrimination with no need for separations or washes (see Landegren U. et al., *Genome Research*, 8:769-

776,1998). The TaqMan assay takes advantage of the 5' nuclease activity of Taq DNA polymerase to digest a DNA probe annealed specifically to the accumulating amplification product. TaqMan probes are labeled with a donor-acceptor dye pair that interacts via fluorescence energy transfer. Cleavage of the TaqMan probe by the advancing polymerase during amplification dissociates the donor dye from the quenching acceptor dye, greatly increasing the donor fluorescence. All reagents necessary to detect two allelic variants can be assembled at the beginning of the reaction and the results are monitored in real time (see Livak et al., *Nature Genetics*, 9:341-342, 1995). In an alternative homogeneous hybridization-based procedure, molecular beacons are used for allele discriminations. Molecular beacons are hairpin-shaped oligonucleotide probes that report the presence of specific nucleic acids in homogeneous solutions. When they bind to their targets they undergo a conformational reorganization that restores the fluorescence of an internally quenched fluorophore (Tyagi et al., *Nature Biotechnology*, 16:49-53, 1998).

The polynucleotides provided herein can be used in hybridization assays for the detection of biallelic marker alleles in biological samples. These probes are characterized in that they preferably comprise between 8 and 50 nucleotides, and in that they are sufficiently complementary to a sequence comprising a biallelic marker of the present invention to hybridize thereto and preferably sufficiently specific to be able to discriminate the targeted sequence for only one nucleotide variation. The GC content in the probes of the invention usually ranges between 10 and 75 %, preferably between 35 and 60 %, and more preferably between 40 and 55 %. The length of these probes can range from 10, 15, 20, or 30 to at least 100 nucleotides, preferably from 10 to 50, more preferably from 18 to 35 nucleotides. A particularly preferred probe is 25 nucleotides in length. Preferably the biallelic marker is within 4 nucleotides of the center of the polynucleotide probe. In particularly preferred probes the biallelic marker is at the center of said polynucleotide. Shorter probes may lack specificity for a target nucleic acid sequence and generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. Longer probes are expensive to produce and can sometimes self-hybridize to form hairpin structures. Methods for the synthesis of oligonucleotide probes have been described above and can be applied to the probes of the present invention.

Preferably the probes of the present invention are labeled or immobilized on a solid support. Labels and solid supports are further described in I. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for example peptide nucleic acids which are disclosed in International Patent Application WO 92/20702, morpholino analogs which are described in U.S. Patents Numbered 5,185,444; 5,034,506 and 5,142,047. The probe may have to be rendered "non-extendable" in that additional dNTPs cannot be

added to the probe. In and of themselves analogs usually are non-extendable and nucleic acid probes can be rendered non-extendable by modifying the 3' end of the probe such that the hydroxyl group is no longer capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified, U.S. Patent Application Serial No. 07/049,061 filed April 19, 1993 describes modifications, which can be used to render a probe non-extendable.

The probes of the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA or Northern hybridization to mRNA. The probes can also be used to detect PCR amplification products. By assaying the hybridization to an allele specific probe, one can detect the presence or absence of a biallelic marker allele in a given sample.

High-Throughput parallel hybridizations in array format are specifically encompassed within "hybridization assays" and are described below.

Hybridization to addressable arrays of oligonucleotides

Hybridization assays based on oligonucleotide arrays rely on the differences in hybridization stability of short oligonucleotides to perfectly matched and mismatched target sequence variants. Efficient access to polymorphism information is obtained through a basic structure comprising high-density arrays of oligonucleotide probes attached to a solid support (the chip) at selected positions. Each DNA chip can contain thousands to millions of individual synthetic DNA probes arranged in a grid-like pattern and miniaturized to the size of a dime.

The chip technology has already been applied with success in numerous cases. For example, the screening of mutations has been undertaken in the BRCA1 gene, in *S. cerevisiae* mutant strains, and in the protease gene of HIV-1 virus (Hacia et al., *Nature Genetics*, 14(4):441-447, 1996; Shoemaker et al., *Nature Genetics*, 14(4):450-456, 1996 ; Kozal et al., *Nature Medicine*, 2:753-759, 1996). Chips of various formats for use in detecting biallelic polymorphisms can be produced on a customized basis by Affymetrix (GeneChip™), Hyseq (HyChip and HyGnostics), and Protogene Laboratories.

In general, these methods employ arrays of oligonucleotide probes that are complementary to target nucleic acid sequence segments from an individual which, target sequences include a polymorphic marker. EP785280 describes a tiling strategy for the detection of single nucleotide polymorphisms. Briefly, arrays may generally be "tiled" for a large number of specific polymorphisms. By "tiling" is generally meant the synthesis of a defined set of oligonucleotide probes which is made up of a sequence complementary to the target sequence of interest, as well as preselected variations of that sequence, e.g., substitution

of one or more given positions with one or more members of the basis set of monomers, i.e. nucleotides. Tiling strategies are further described in PCT application No. WO 95/11995. In a particular aspect, arrays are tiled for a number of specific, identified biallelic marker sequences. In particular the array is tiled to include a number of detection blocks, each detection block being specific for a specific biallelic marker or a set of biallelic markers. For example, a detection block may be tiled to include a number of probes, which span the sequence segment that includes a specific polymorphism. To ensure probes that are complementary to each allele, the probes are synthesized in pairs differing at the biallelic marker. In addition to the probes differing at the polymorphic base, monosubstituted probes are also generally tiled within the detection block. These monosubstituted probes have bases at and up to a certain number of bases in either direction from the polymorphism, substituted with the remaining nucleotides (selected from A, T, G, C and U). Typically the probes in a tiled detection block will include substitutions of the sequence positions up to and including those that are 5 bases away from the biallelic marker. The monosubstituted probes provide internal controls for the tiled array, to distinguish actual hybridization from artefactual cross-hybridization. Upon completion of hybridization with the target sequence and washing of the array, the array is scanned to determine the position on the array to which the target sequence hybridizes. The hybridization data from the scanned array is then analyzed to identify which allele or alleles of the biallelic marker are present in the sample. Hybridization and scanning may be carried out as described in PCT application No. WO 92/10092 and WO 95/11995 and US patent No. 5,424,186.

Thus, in some embodiments, the chips may comprise an array of nucleic acid sequences of fragments of about 15 nucleotides in length. In further embodiments, the chip may comprise an array including at least one of the sequences selected from the group consisting of SEQ ID No. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 and the sequences complementary thereto, or a fragment thereof at least about 8 consecutive nucleotides, preferably 10, 15, 20, more preferably least 30, 35, 43, 44, 45, 46 or 47 consecutive nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID. In some embodiments, the chip may comprise an array of at least 2, 3, 4, 5, 6, 7, 8 or more of these polynucleotides of the invention. Solid supports and polynucleotides of the present invention attached to solid supports are further described in I.

5) Integrated Systems

Another technique, which may be used to analyze polymorphisms, includes multicomponent integrated systems, which miniaturize and compartmentalize processes such as PCR and capillary electrophoresis reactions in a single functional device. An example of

such technique is disclosed in US patent 5,589,136, which describes the integration of PCR amplification and capillary electrophoresis in chips.

Integrated systems can be envisaged mainly when microfluidic systems are used. These systems comprise a pattern of microchannels designed onto a glass, silicon, quartz, or plastic wafer included on a microchip. The movements of the samples are controlled by electric, electroosmotic or hydrostatic forces applied across different areas of the microchip. For genotyping biallelic markers, the microfluidic system may integrate nucleic acid amplification, microsequencing, capillary electrophoresis and a detection method such as laser-induced fluorescence detection.

IV. Methods Of Genetic Analysis Using The Biallelic Markers Of The Present Invention

Different methods are available for the genetic analysis of complex traits (see Lander and Schork, *Science*, 265, 2037-2048, 1994). The search for disease-susceptibility genes is conducted using two main methods: the linkage approach in which evidence is sought for cosegregation between a locus and a putative trait locus using family studies, and the association approach in which evidence is sought for a statistically significant association between an allele and a trait or a trait causing allele (Khoury J. et al., *Fundamentals of Genetic Epidemiology, Oxford University Press, NY*, 1993). In general, the biallelic markers of the present invention find use in any method known in the art to demonstrate a statistically significant correlation between a genotype and a phenotype. The biallelic markers may be used in parametric and non-parametric linkage analysis methods. Preferably, the biallelic markers of the present invention are used to identify genes associated with detectable traits using association studies, an approach which does not require the use of affected families and which permits the identification of genes associated with complex and sporadic traits.

The genetic analysis using the biallelic markers of the present invention may be conducted on any scale. The whole set of biallelic markers of the present invention or any subset of biallelic markers of the present invention may be used. In some embodiments a subset of biallelic markers corresponding to one or several candidate genes may be used. In other embodiments a subset of biallelic markers corresponding to candidate genes from a particular disease pathway may be used. Alternatively, a subset of biallelic markers of the present invention localised on a specific chromosome segment may be used. Further, any set of genetic markers including a biallelic marker of the present invention may be used. A set of biallelic polymorphisms that, could be used as genetic markers in combination with the biallelic markers of the present invention, has been described in WO 98/20165. As mentioned above, it should be noted that the biallelic markers of the present invention may be included in any complete or partial genetic map of the human genome. These different uses are specifically contemplated in the present invention and claims.

IV.A. Linkage analysis

Linkage analysis is based upon establishing a correlation between the transmission of genetic markers and that of a specific trait throughout generations within a family. Thus, the aim of linkage analysis is to detect marker loci that show cosegregation with a trait of interest in pedigrees.

Parametric methods

When data are available from successive generations there is the opportunity to study the degree of linkage between pairs of loci. Estimates of the recombination fraction enable loci to be ordered and placed onto a genetic map. With loci that are genetic markers, a genetic map can be established, and then the strength of linkage between markers and traits can be calculated and used to indicate the relative positions of markers and genes affecting those traits (Weir, B.S., *Genetic data Analysis II: Methods for Discrete population genetic Data, Sinauer Assoc., Inc., Sunderland, MA, USA,* 1996). The classical method for linkage analysis is the logarithm of odds (lod) score method (see Morton N.E., *Am.J. Hum.Genet.*, 7:277-318, 1955; Ott J., *Analysis of Human Genetic Linkage, John Hopkins University Press, Baltimore,* 1991). Calculation of lod scores requires specification of the mode of inheritance for the disease (parametric method). Generally, the length of the candidate region identified using linkage analysis is between 2 and 20Mb. Once a candidate region is identified as described above, analysis of recombinant individuals using additional markers allows further delineation of the candidate region. Linkage analysis studies have generally relied on the use of a maximum of 5,000 microsatellite markers, thus limiting the maximum theoretical attainable resolution of linkage analysis to about 600 kb on average.

Linkage analysis has been successfully applied to map simple genetic traits that show clear Mendelian inheritance patterns and which have a high penetrance (i.e., the ratio between the number of trait positive carriers of allele *a* and the total number of *a* carriers in the population). However, parametric linkage analysis suffers from a variety of drawbacks. First, it is limited by its reliance on the choice of a genetic model suitable for each studied trait. Furthermore, as already mentioned, the resolution attainable using linkage analysis is limited, and complementary studies are required to refine the analysis of the typical 2Mb to 20Mb regions initially identified through linkage analysis. In addition, parametric linkage analysis approaches have proven difficult when applied to complex genetic traits, such as those due to the combined action of multiple genes and/or environmental factors. It is very difficult to model these factors adequately in a lod score analysis. In such cases, too large an effort and cost are needed to recruit the adequate number of affected families required for applying linkage analysis to these situations, as recently discussed by Risch, N. and Merikangas, K. (*Science*, 273:1516-1517, 1996).

Non-parametric methods

The advantage of the so-called non-parametric methods for linkage analysis is that they do not require specification of the mode of inheritance for the disease, they tend to be more useful for the analysis of complex traits. In non-parametric methods, one tries to prove that the inheritance pattern of a chromosomal region is not consistent with random Mendelian segregation by showing that affected relatives inherit identical copies of the region more often than expected by chance. Affected relatives should show excess "allele sharing" even in the presence of incomplete penetrance and polygenic inheritance. In non-parametric linkage analysis the degree of agreement at a marker locus in two individuals can be measured either by the number of alleles identical by state (IBS) or by the number of alleles identical by descent (IBD). Affected sib pair analysis is a well-known special case and is the simplest form of these methods.

The biallelic markers of the present invention may be used in both parametric and non-parametric linkage analysis. Preferably biallelic markers may be used in non-parametric methods which allow the mapping of genes involved in complex traits. The biallelic markers of the present invention may be used in both IBD- and IBS- methods to map genes affecting a complex trait. In such studies, taking advantage of the high density of biallelic markers, several adjacent biallelic marker loci may be pooled to achieve the efficiency attained by multi-allelic markers (Zhao et al., *Am. J. Hum. Genet.*, 63:225-240, 1998).

However, both parametric and non-parametric linkage analysis methods analyse affected relatives, they tend to be of limited value in the genetic analysis of drug responses or in the analysis of side effects to treatments. This type of analysis is impractical in such cases due to the lack of availability of familial cases. In fact, the likelihood of having more than one individual in a family being exposed to the same drug at the same time is extremely low.

IV.B. Population Association studies

The present invention comprises methods for identifying one or several genes among a set of candidate genes that are associated with a detectable trait using the biallelic markers of the present invention. In one embodiment the present invention comprises methods to detect an association between a biallelic marker allele or a biallelic marker haplotype and a trait. Further, the invention comprises methods to identify a trait causing allele in linkage disequilibrium with any biallelic marker allele of the present invention.

As described above, alternative approaches can be employed to perform association studies: genome-wide association studies, candidate region association studies and candidate gene association studies. In a preferred embodiment, the biallelic markers of the present invention are used to perform candidate gene association studies. Further, the biallelic markers of the present invention may be incorporated in any map of genetic markers of the

human genome in order to perform genome-wide association studies. Methods to generate a high-density map of biallelic markers has been described in US Provisional Patent application serial number 60/082,614. The biallelic markers of the present invention may further be incorporated in any map of a specific candidate region of the genome (a specific chromosome or a specific chromosomal segment for example).

As mentioned above, association studies may be conducted within the general population and are not limited to studies performed on related individuals in affected families. Association studies are extremely valuable as they permit the analysis of sporadic or multifactor traits. Moreover, association studies represent a powerful method for fine-scale mapping enabling much finer mapping of trait causing alleles than linkage studies. Studies based on pedigrees often only narrow the location of the trait causing allele. Association studies using the biallelic markers of the present invention can therefore be used to refine the location of a trait causing allele in a candidate region identified by Linkage Analysis methods. Moreover, once a chromosome segment of interest has been identified, the presence of a candidate gene such as a candidate gene of the present invention, in the region of interest can provide a shortcut to the identification of the trait causing allele. Biallelic markers of the present invention can be used to demonstrate that a candidate gene is associated with a trait. Such uses are specifically contemplated in the present invention and claims.

1) Determining the frequency of a biallelic marker allele or of a biallelic marker haplotype in a population

Association studies explore the relationships among frequencies for sets of alleles between loci.

Determining the frequency of an allele in a population

Allelic frequencies of the biallelic markers in a population can be determined using one of the methods described above under the heading "Methods for genotyping an individual for biallelic markers", or any genotyping procedure suitable for this intended purpose. Genotyping pooled samples or individual samples can determine the frequency of a biallelic marker allele in a population. One way to reduce the number of genotypings required is to use pooled samples. A major obstacle in using pooled samples is in terms of accuracy and reproducibility for determining accurate DNA concentrations in setting up the pools. Genotyping individual samples provides higher sensitivity, reproducibility and accuracy and; is the preferred method used in the present invention. Preferably, each individual is genotyped separately and simple gene counting is applied to determine the frequency of an allele of a biallelic marker or of a genotype in a given population.

Determining the frequency of a haplotype in a population

The gametic phase of haplotypes is unknown when diploid individuals are

heterozygous at more than one locus. Using genealogical information in families gametic phase can sometimes be inferred (Perlin et al., *Am. J. Hum. Genet.*, 55:777-787, 1994). When no genealogical information is available different strategies may be used. One possibility is that the multiple-site heterozygous diploids can be eliminated from the analysis, keeping only the homozygotes and the single-site heterozygote individuals, but this approach might lead to a possible bias in the sample composition and the underestimation of low-frequency haplotypes. Another possibility is that single chromosomes can be studied independently, for example, by asymmetric PCR amplification (see Newton et al., *Nucleic Acids Res.*, 17:2503-2516, 1989; Wu et al., *Proc. Natl. Acad. Sci. USA*, 86:2757, 1989) or by isolation of single chromosome by limit dilution followed by PCR amplification (see Ruano et al., *Proc. Natl. Acad. Sci. USA*, 87:6296-6300, 1990). Further, a sample may be haplotyped for sufficiently close biallelic markers by double PCR amplification of specific alleles (Sarkar, G. and Sommer S.S., *Biotechniques*, 1991). These approaches are not entirely satisfying either because of their technical complexity, the additional cost they entail, their lack of generalisation at a large scale, or the possible biases they introduce. To overcome these difficulties, an algorithm to infer the phase of PCR-amplified DNA genotypes introduced by Clark A.G. (*Mol. Biol. Evol.*, 7:111-122, 1990) may be used. Briefly, the principle is to start filling a preliminary list of haplotypes present in the sample by examining unambiguous individuals, that is, the complete homozygotes and the single-site heterozygotes. Then other individuals in the same sample are screened for the possible occurrence of previously recognised haplotypes. For each positive identification, the complementary haplotype is added to the list of recognised haplotypes, until the phase information for all individuals is either resolved or identified as unresolved. This method assigns a single haplotype to each multiheterozygous individual, whereas several haplotypes are possible when there are more than one heterozygous site. Alternatively, one can use methods estimating haplotype frequencies in a population without assigning haplotypes to each individual. Preferably, a method based on an expectation-maximization (EM) algorithm (Dempster et al., *J. R. Stat. Soc.*, 39B: 1-38, 1977) leading to maximum-likelihood estimates of haplotype frequencies under the assumption of Hardy-Weinberg proportions (random mating) is used (see Excoffier L. and Slatkin M., *Mol. Biol. Evol.*, 12(5): 921-927, 1995). The EM algorithm is a generalised iterative maximum-likelihood approach to estimation that is useful when data are ambiguous and/or incomplete. The EM algorithm is used to resolve heterozygotes into haplotypes. Haplotype estimations are further described below under the heading "Statistical methods". Any other method known in the art to determine or to estimate the frequency of a haplotype in a population may also be used.

2) Linkage Disequilibrium analysis

Linkage disequilibrium is the non-random association of alleles at two or more loci and represents a powerful tool for mapping genes involved in disease traits (see Ajioka R.S. et al., *Am. J. Hum. Genet.*, 60:1439-1447, 1997). Biallelic markers, because they are densely spaced in the human genome and can be genotyped in more numerous numbers than other types of genetic markers (such as RFLP or VNTR markers), are particularly useful in genetic analysis based on linkage disequilibrium. The biallelic markers of the present invention may be used in any linkage disequilibrium analysis method known in the art.

Briefly, when a disease mutation is first introduced into a population (by a new mutation or the immigration of a mutation carrier), it necessarily resides on a single chromosome and thus on a single "background" or "ancestral" haplotype of linked markers. Consequently, there is complete disequilibrium between these markers and the disease mutation: one finds the disease mutation only in the presence of a specific set of marker alleles. Through subsequent generations recombinations occur between the disease mutation and these marker polymorphisms, and the disequilibrium gradually dissipates. The pace of this dissipation is a function of the recombination frequency, so the markers closest to the disease gene will manifest higher levels of disequilibrium than those that are further away. When not broken up by recombination, "ancestral" haplotypes and linkage disequilibrium between marker alleles at different loci can be tracked not only through pedigrees but also through populations. Linkage disequilibrium is usually seen as an association between one specific allele at one locus and another specific allele at a second locus.

The pattern or curve of disequilibrium between disease and marker loci is expected to exhibit a maximum that occurs at the disease locus. Consequently, the amount of linkage disequilibrium between a disease allele and closely linked genetic markers may yield valuable information regarding the location of the disease gene. For fine-scale mapping of a disease locus, it is useful to have some knowledge of the patterns of linkage disequilibrium that exist between markers in the studied region. As mentioned above the mapping resolution achieved through the analysis of linkage disequilibrium is much higher than that of linkage studies. The high density of biallelic markers combined with linkage disequilibrium analysis provides powerful tools for fine-scale mapping. Different methods to calculate linkage disequilibrium are described below under the heading "Statistical Methods".

3) Population-based case-control studies of trait-marker associations

As mentioned above, the occurrence of pairs of specific alleles at different loci on the same chromosome is not random and the deviation from random is called linkage disequilibrium. Association studies focus on population frequencies and rely on the phenomenon of linkage disequilibrium. If a specific allele in a given gene is directly involved in causing a particular trait, its frequency will be statistically increased in an affected (trait

positive) population, when compared to the frequency in a trait negative population or in a random control population. As a consequence of the existence of linkage disequilibrium, the frequency of all other alleles present in the haplotype carrying the trait-causing allele will also be increased in trait positive individuals compared to trait negative individuals or random controls. Therefore, association between the trait and any allele (specifically a biallelic marker allele) in linkage disequilibrium with the trait-causing allele will suffice to suggest the presence of a trait-related gene in that particular region. Case-control populations can be genotyped for biallelic markers to identify associations that narrowly locate a trait causing allele. As any marker in linkage disequilibrium with one given marker associated with a trait will be associated with the trait. Linkage disequilibrium allows the relative frequencies in case-control populations of a limited number of genetic polymorphisms (specifically biallelic markers) to be analysed as an alternative to screening all possible functional polymorphisms in order to find trait-causing alleles. Association studies compare the frequency of marker alleles in unrelated case-control populations, and represent powerful tools for the dissection of complex traits.

Case-control populations (inclusion criteria)

Population-based association studies do not concern familial inheritance but compare the prevalence of a particular genetic marker, or a set of markers, in case-control populations. They are case-control studies based on comparison of unrelated case (affected or trait positive) individuals and unrelated control (unaffected or trait negative or random) individuals. Preferably the control group is composed of unaffected or trait negative individuals. Further, the control group is ethnically matched to the case population. Moreover, the control group is preferably matched to the case-population for the main known confusion factor for the trait under study (for example age-matched for an age-dependent trait). Ideally, individuals in the two samples are paired in such a way that they are expected to differ only in their disease status. In the following "trait positive population", "case population" and "affected population" are used interchangeably.

An important step in the dissection of complex traits using association studies is the choice of case-control populations (see Lander and Schork, *Science*, 265, 2037-2048, 1994). A major step in the choice of case-control populations is the clinical definition of a given trait or phenotype. Any genetic trait may be analysed by the association method proposed here by carefully selecting the individuals to be included in the trait positive and trait negative phenotypic groups. Four criteria are often useful: clinical phenotype, age at onset, family history and severity. The selection procedure for continuous or quantitative traits (such as blood pressure for example) involves selecting individuals at opposite ends of the phenotype distribution of the trait under study, so as to include in these trait positive and trait negative

populations individuals with non-overlapping phenotypes. Preferably, case-control populations consist of phenotypically homogeneous populations. Trait positive and trait negative populations consist of phenotypically uniform populations of individuals representing each between 1 and 98%, preferably between 1 and 80%, more preferably between 1 and 50%, and more preferably between 1 and 30%, most preferably between 1 and 20% of the total population under study, and selected among individuals exhibiting non-overlapping phenotypes. The clearer the difference between the two trait phenotypes, the greater the probability of detecting an association with biallelic markers. The selection of those drastically different but relatively uniform phenotypes enables efficient comparisons in association studies and the possible detection of marked differences at the genetic level, provided that the sample sizes of the populations under study are significant enough.

In preferred embodiments, a first group of between 50 and 300 trait positive individuals, preferably about 100 individuals, are recruited according to their phenotypes. A similar number of trait negative individuals are included in such studies.

Association analysis

The general strategy to perform association studies using biallelic markers derived from a region carrying a candidate gene is to scan two groups of individuals (case-control populations) in order to measure and statistically compare the allele frequencies of the biallelic markers of the present invention in both groups.

If a statistically significant association with a trait is identified for at least one or more of the analysed biallelic markers, one can assume that: either the associated allele is directly responsible for causing the trait (the associated allele is the trait causing allele), or more likely the associated allele is in linkage disequilibrium with the trait causing allele. The specific characteristics of the associated allele with respect to the candidate gene function usually gives further insight into the relationship between the associated allele and the trait (causal or in linkage disequilibrium). If the evidence indicates that the associated allele within the candidate gene is most probably not the trait causing allele but is in linkage disequilibrium with the real trait causing allele, then the trait causing allele can be found by sequencing the vicinity of the associated marker.

Association studies are usually run in two successive steps. In a first phase, the frequencies of a reduced number of biallelic markers from one or several candidate genes are determined in the trait positive and trait negative populations. In a second phase of the analysis, the identity of the candidate gene and the position of the genetic loci responsible for the given trait is further refined using a higher density of markers from the relevant region. However, if the candidate gene under study is relatively small in length, as it is the case for many of the candidate genes analysed included in the present invention, a single phase may be

sufficient to establish significant associations.

Haplotype analysis

As described above, when a chromosome carrying a disease allele first appears in a population as a result of either mutation or migration, the mutant allele necessarily resides on a chromosome having a set of linked markers: the ancestral haplotype. This haplotype can be tracked through populations and its statistical association with a given trait can be analysed. Complementing single point (allelic) association studies with multi-point association studies also called haplotype studies increases the statistical power of association studies. Thus, a haplotype association study allows one to define the frequency and the type of the ancestral carrier haplotype. A haplotype analysis is important in that it increases the statistical power of an analysis involving individual markers.

In a first stage of a haplotype frequency analysis, the frequency of the possible haplotypes based on various combinations of the identified biallelic markers of the invention is determined. The haplotype frequency is then compared for distinct populations of trait positive and control individuals. The number of trait positive individuals, which should be, subjected to this analysis to obtain statistically significant results usually ranges between 30 and 300, with a preferred number of individuals ranging between 50 and 150. The same considerations apply to the number of unaffected individuals (or random control) used in the study. The results of this first analysis provide haplotype frequencies in case-control populations, for each evaluated haplotype frequency a p-value and an odd ratio are calculated. If a statistically significant association is found the relative risk for an individual carrying the given haplotype of being affected with the trait under study can be approximated.

Interaction Analysis

The biallelic markers of the present invention may also be used to identify patterns of biallelic markers associated with detectable traits resulting from polygenic interactions. The analysis of genetic interaction between alleles at unlinked loci requires individual genotyping using the techniques described herein. The analysis of allelic interaction among a selected set of biallelic markers with appropriate level of statistical significance can be considered as a haplotype analysis. Interaction analysis consists in stratifying the case-control populations with respect to a given haplotype for the first loci and performing a haplotype analysis with the second loci with each subpopulation.

Statistical methods used in association studies are further described below in IV.C.

4) Testing for linkage in the presence of association

The biallelic markers of the present invention may further be used in TDT (transmission/disequilibrium test). TDT tests for both linkage and association and is not affected by population stratification. TDT requires data for affected individuals and their

parents or data from unaffected sibs instead of from parents (see Spielmann S. et al., *Am. J. Hum. Genet.*, 52:506-516, 1993; Schaid D.J. et al., *Genet. Epidemiol.*,13:423-450, 1996, Spielmann S. and Ewens W.J., *Am. J. Hum. Genet.*, 62:450-458, 1998). Such combined tests generally reduce the false – positive errors produced by separate analyses.

IV.C. Statistical methods

In general, any method known in the art to test whether a trait and a genotype show a statistically significant correlation may be used.

1) Methods in linkage analysis

Statistical methods and computer programs useful for linkage analysis are well-known to those skilled in the art (see Terwilliger J.D. and Ott J., *Handbook of Human Genetic Linkage, John Hopkins University Press, London,* 1994; Ott J., *Analysis of Human Genetic Linkage, John Hopkins University Press, Baltimore,* 1991).

2) Methods to estimate haplotype frequencies in a population

As described above, when genotypes are scored, it is often not possible to distinguish heterozygotes so that haplotype frequencies cannot be easily inferred. When the gametic phase is not known, haplotype frequencies can be estimated from the multilocus genotypic data. Any method known to person skilled in the art can be used to estimate haplotype frequencies (see Lange K., *Mathematical and Statistical Methods for Genetic Analysis, Springer, New York,* 1997; Weir, B.S., *Genetic data Analysis II: Methods for Discrete population genetic Data, Sinauer Assoc., Inc., Sunderland, MA, USA,* 1996) Preferably, maximum-likelihood haplotype frequencies are computed using an Expectation- Maximization (EM) algorithm (see Dempster et al., *J. R. Stat. Soc.*, 39B:1-38, 1977; Excoffier L. and Slatkin M., *Mol. Biol. Evol.*, 12(5): 921-927, 1995). This procedure is an iterative process aiming at obtaining maximum-likelihood estimates of haplotype frequencies from multi-locus genotype data when the gametic phase is unknown. Haplotype estimations are usually performed by applying the EM algorithm using for example the EM-HAPLO program (Hawley M.E. et al., *Am. J. Phys. Anthropol.*, 18:104, 1994) or the Arlequin program (Schneider et al., *Arlequin: a software for population genetics data analysis*, University of Geneva, 1997). The EM algorithm is a generalised iterative maximum likelihood approach to estimation and is briefly described below.

In the following part of this text, phenotypes will refer to multi-locus genotypes with unknown phase. Genotypes will refer to known-phase multi-locus genotypes.

Suppose a sample of N unrelated individuals typed for K markers. The data observed are the unknown-phase K-locus phenotypes that can categorised in F different phenotypes. Suppose that we have H underlying possible haplotypes (in case of K biallelic markers, $H=2^K$).

For phenotype j, suppose that c_j genotypes are possible. We thus have the following equation

$$P_j = \sum_{i=1}^{c_j} pr(genotype_i) = \sum_{i=1}^{c_j} pr(h_k, h_l) \qquad \text{Equation 1}$$

where Pj is the probability of the phenotype j, h_k and h_l are the two haplotypes constituent the genotype i. Under the Hardy-Weinberg equilibrium, $pr(h_k h_l)$ becomes :

$$pr(h_k, h_l) = pr(h_k)^2 \text{ if } h_k = h_l,\ pr(h_k, h_l) = 2pr(h_k).pr(h_l) \text{ if } h_k \neq h_l.$$

Equation 2

The successive steps of the E-M algorithm can be described as follows:

Starting with initial values of the of haplotypes frequencies, noted $p_1^{(0)}, p_2^{(0)}, \ldots p_H^{(0)}$, these initial values serve to estimate the genotype frequencies (Expectation step) and then estimate another set of haplotype frequencies (Maximisation step), noted $p_1^{(1)}, p_2^{(1)}, \ldots p_H^{(1)}$, these two steps are iterated until changes in the sets of haplotypes frequency are very small.

A stop criterion can be that the maximum difference between haplotype frequencies between two iterations is less than 10^{-7}. These values can be adjusted according to the desired precision of estimations.

In details, at a given iteration s, the Expectation step consists in calculating the genotypes frequencies by the following equation:

$$pr(genotype_i)^{(s)} = pr(phenotype_j).pr(genotype_i | phenotype_j)^{(s)}$$
$$= \frac{n_j}{N} \cdot \frac{pr(h_k, h_l)^{(s)}}{P_j^{(s)}}$$

Equation 3

where genotype i occurs in phenotype j, and where h_k and h_l constitute genotype i. Each probability is derived according to eq.1, and eq.2 described above.

Then the Maximisation step simply estimates another set of haplotype frequencies given the genotypes frequencies. This approach is also known as gene-counting method (Smith, *Ann. Hum. Genet.*, 21:254-276, 1957).

$$p_t^{(s+1)} = \frac{1}{2} \sum_{j=1}^{F} \sum_{i=1}^{c_j} \delta_{it} . pr(genotype_l)^{(s)} \qquad \text{Equation 4}$$

Where δ_{it} is an indicator variable which count the number of time haplotype t in genotype i. It takes the values of 0, 1 or 2.

To ensure that the estimation finally obtained is the maximum-likelihood estimation several values of departures are required. The estimations obtained are compared and if they are different the estimations leading to the best likelihood are kept.

3) Methods to calculate linkage disequilibrium between markers

A number of methods can be used to calculate linkage disequilibrium between any two genetic positions, in practice linkage disequilibrium is measured by applying a statistical association test to haplotype data taken from a population.

Linkage disequilibrium between any pair of biallelic markers comprising at least one of the biallelic markers of the present invention (M_i, M_j) having alleles (a_i/b_i) at marker M_i and alleles (a_j/b_j) at marker M_j can be calculated for every allele combination (a_i,a_j ; a_i,b_j; b_i,a_j and b_i,b_j), according to the Piazza formula :

$\Delta_{aiaj} = \sqrt{\theta 4} - \sqrt{(\theta 4 + \theta 3)(\theta 4 + \theta 2)}$, where :

$\theta 4$= - - = frequency of genotypes not having allele a_i at M_i and not having allele a_j at M_j

$\theta 3$= - + = frequency of genotypes not having allele a_i at M_i and having allele a_j at M_j

$\theta 2$= + - = frequency of genotypes having allele a_i at M_i and not having allele a_j at M_j

Linkage disequilibrium (LD) between pairs of biallelic markers (M_i, M_j) can also be calculated for every allele combination (ai,aj; ai,bj ; b_i,a_j and b_i,b_j), according to the maximum-likelihood estimate (MLE) for delta (the composite genotypic disequilibrium coefficient), as described by Weir (Weir B.S., *Genetic Data Analysis*, *Sinauer Ass. Eds,* 1996). The MLE for the composite linkage disequilibrium is:

$D_{aiaj} = (2n_1 + n_2 + n_3 + n_4/2)/N - 2(pr(a_i).pr(a_j))$

Where n_1 = Σ phenotype (a_i/a_i, a_j/a_j), n_2 = Σ phenotype (a_i/a_i, a_j/b_j), n_3= Σ phenotype (a_i/b_i, a_j/a_j), n4= Σ phenotype (a_i/b_i, a_j/b_j) and N is the number of individuals in the sample.

This formula allows linkage disequilibrium between alleles to be estimated when only genotype, and not haplotype, data are available.

Another means of calculating the linkage disequilibrium between markers is as follows. For a couple of biallelic markers, M_i (a_i/b_i) and M_j (a_j/b_j), fitting the Hardy-Weinberg equilibrium, one can estimate the four possible haplotype frequencies in a given population according to the approach described above.

The estimation of gametic disequilibrium between *ai* and *aj* is simply:

$$D_{aiaj} = pr(haplotype(a_i, a_j)) - pr(a_i).pr(a_j).$$

Where $pr(a_i)$ is the probability of allele a_i and pr(a_j) is the probability of allele a_j and where $pr(haplotype\ (a_i, a_j))$ is estimated as in Equation 3 above.

For a couple of biallelic marker only one measure of disequilibrium is necessary to describe the association between M_i and M_j.

Then a normalised value of the above is calculated as follows:

$$D'_{aiaj} = D_{aiaj} / \max(-pr(a_i).pr(a_j), -pr(b_i).pr(b_j)) \quad \text{with } D_{aiaj}<0$$

$$D'_{aiaj} = D_{aiaj} / \max(pr(b_i).pr(a_j), pr(a_i).pr(b_j)) \quad \text{with } D_{aiaj}>0$$

The skilled person will readily appreciate that other LD calculation methods can be used without undue experimentation.

Linkage disequilibrium among a set of biallelic markers having an adequate heterozygosity rate can be determined by genotyping between 50 and 1000 unrelated individuals, preferably between 75 and 200, more preferably around 100.

4) Testing for association

Methods for determining the statistical significance of a correlation between a phenotype and a genotype, in this case an allele at a biallelic marker or a haplotype made up of such alleles, may be determined by any statistical test known in the art and with any accepted threshold of statistical significance being required. The application of particular methods and thresholds of significance are well with in the skill of the ordinary practitioner of the art.

Testing for association is performed by determining the frequency of a biallelic marker allele in case and control populations and comparing these frequencies with a statistical test to determine if their is a statistically significant difference in frequency which would indicate a correlation between the trait and the biallelic marker allele under study. Similarly, a haplotype analysis is performed by estimating the frequencies of all possible haplotypes for a given set of biallelic markers in case and control populations, and comparing these frequencies with a statistical test to determine if their is a statistically significant correlation between the haplotype and the phenotype (trait) under study. Any statistical tool useful to test for a statistically significant association between a genotype and a phenotype may be used. Preferably the statistical test employed is a chi-square test with one degree of freedom. A p-value is calculated (the p-value is the probability that a statistic as large or larger than the observed one would occur by chance).

Statistical significance

In preferred embodiments, significance for diagnosis purposes, either as a positive basis for further diagnostic tests or as a preliminary starting point for early preventive therapy, the p value related to a biallelic marker association is preferably about 1 x 10-2 or less, more preferably about 1 x 10-4 or less, for a single biallelic marker analysis and about 1 x 10-3 or less, still more preferably 1 x 10-6 or less and most preferably of about 1 x 10-8 or less, for a haplotype analysis involving several markers. These values are believed to be applicable to any association studies involving single or multiple marker combinations.

The skilled person can use the range of values set forth above as a starting point in order to carry out association studies with biallelic markers of the present invention. In doing so, significant associations between the biallelic markers of the present invention and diseases can be revealed.

Phenotypic permutation

In order to confirm the statistical significance of the first stage haplotype analysis described above, it might be suitable to perform further analyses in which genotyping data from case-control individuals are pooled and randomised with respect to the trait phenotype. Each individual genotyping data is randomly allocated to two groups, which contain the same number of individuals as the case-control populations used to compile the data obtained in the first stage. A second stage haplotype analysis is preferably run on these artificial groups, preferably for the markers included in the haplotype of the first stage analysis showing the highest relative risk coefficient. This experiment is reiterated preferably at least between 100 and 10000 times. The repeated iterations allow the determination of the percentage of obtained haplotypes with a significant p-value level.

Assessment of statistical association

To address the problem of false positives similar analysis may be performed with the same case-control populations in random genomic regions. Results in random regions and the candidate region are compared as described in US Provisional Patent Application entitled "Methods, software and apparati for identifying genomic regions harbouring a gene associated with a detectable trait".

5) Evaluation of risk factors

The association between a risk factor (in genetic epidemiology the risk factor is the presence or the absence of a certain allele or haplotype at marker loci) and a disease is measured by the odds ratio (OR) and by the relative risk (RR). If $P(R^+)$ is the probability of developing the disease for individuals with R and $P(R^-)$ is the probability for individuals without the risk factor, then the relative risk is simply the ratio of the two probabilities, that is:

$$RR = P(R^+)/P(R^-)$$

In case-control studies, direct measures of the relative risk cannot be obtained because of the sampling design. However, the odds ratio allows a good approximation of the relative risk for low-incidence diseases and can be calculated:

$$OR = \left[\frac{F^+}{1-F^+}\right] \Big/ \left[\frac{F^-}{(1-F^-)}\right]$$

F^+ is the frequency of the exposure to the risk factor in cases and F^- is the frequency of the exposure to the risk factor in controls. F^+ and F^- are calculated using the allelic or haplotype frequencies of the study and further depend on the underlying genetic model (dominant, recessive, additive...).

One can further estimate the attributable risk (AR) which describes the proportion of individuals in a population exhibiting a trait due to a given risk factor. This measure is

important in quantitating the role of a specific factor in disease etiology and in terms of the public health impact of a risk factor. The public health relevance of this measure lies in estimating the proportion of cases of disease in the population that could be prevented if the exposure of interest were absent. AR is determined as follows:

$$AR = P_E (RR-1) / (P_E (RR-1)+1)$$

AR is the risk attributable to a biallelic marker allele or a biallelic marker haplotype. P_E is the frequency of exposure to an allele or a haplotype within the population at large; and RR is the relative risk which, is approximated with the odds ratio when the trait under study has a relatively low incidence in the general population.

IV.F. Identification Of Biallelic Markers In Linkage Disequilibrium With The Biallelic Markers of the Invention

Once a first biallelic marker has been identified in a genomic region of interest, the practitioner of ordinary skill in the art, using the teachings of the present invention, can easily identify additional biallelic markers in linkage disequilibrium with this first marker. As mentioned before any marker in linkage disequilibrium with a first marker associated with a trait will be associated with the trait. Therefore, once an association has been demonstrated between a given biallelic marker and a trait, the discovery of additional biallelic markers associated with this trait is of great interest in order to increase the density of biallelic markers in this particular region. The causal gene or mutation will be found in the vicinity of the marker or set of markers showing the highest correlation with the trait.

Identification of additional markers in linkage disequilibrium with a given marker involves: (a) amplifying a genomic fragment comprising a first biallelic marker from a plurality of individuals; (b) identifying of second biallelic markers in the genomic region harboring said first biallelic marker; (c) conducting a linkage disequilibrium analysis between said first biallelic marker and second biallelic markers; and (d) selecting said second biallelic markers as being in linkage disequilibrium with said first marker. Subcombinations comprising steps (b) and (c) are also contemplated.

Methods to identify biallelic markers and to conduct linkage disequilibrium analysis are described herein and can be carried out by the skilled person without undue experimentation. The present invention then also concerns biallelic markers which are in linkage disequilibrium with any of the specific biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 and which are expected to present similar characteristics in terms of their respective association with a given trait.

Example 5 illustrates the measurement of linkage disequilibrium between a publicly known biallelic marker, the "ApoE Site A", located within the Alzheimer's related ApoE gene, and other biallelic markers randomly derived from the genomic region containing the

ApoE gene.

IV.G. Identification Of Functional Mutations

Once a positive association is confirmed with a biallelic marker of the present invention, the associated candidate gene can be scanned for mutations by comparing the sequences of a selected number of trait positive and trait negative individuals. In a preferred embodiment, functional regions such as exons and splice sites, promoters and other regulatory regions of the candidate gene are scanned for mutations. Preferably, trait positive individuals carry the haplotype shown to be associated with the trait and trait negative individuals do not carry the haplotype or allele associated with the trait. The mutation detection procedure is essentially similar to that used for biallelic site identification.

The method used to detect such mutations generally comprises the following steps: (a) amplification of a region of the candidate gene comprising a biallelic marker or a group of biallelic markers associated with the trait from DNA samples of trait positive patients and trait negative controls; (b) sequencing of the amplified region; (c) comparison of DNA sequences from trait-positive patients and trait-negative controls; and (d) determination of mutations specific to trait-positive patients. Subcombinations which comprise steps (b) and (c) are specifically contemplated.

It is preferred that candidate polymorphisms be then verified by screening a larger population of cases and controls by means of any genotyping procedure such as those described herein, preferably using a microsequencing technique in an individual test format. Polymorphisms are considered as candidate mutations when present in cases and controls at frequencies compatible with the expected association results.

V. Biallelic Markers Of The Invention In Methods Of Genetic Diagnostics

The biallelic markers of the present invention can also be used to develop diagnostics tests capable of identifying individuals who express a detectable trait as the result of a specific genotype or individuals whose genotype places them at risk of developing a detectable trait at a subsequent time. The trait analyzed using the present diagnostics may be any detectable trait, including a disease, a response to an agent acting on a disease, or side effects to an agent acting on a disease.

The diagnostic techniques of the present invention may employ a variety of methodologies to determine whether a test subject has a biallelic marker pattern associated with an increased risk of developing a detectable trait or whether the individual suffers from a detectable trait as a result of a particular mutation, including methods which enable the analysis of individual chromosomes for haplotyping, such as family studies, single sperm DNA analysis or somatic hybrids.

The present invention provides diagnostic methods to determine whether an individual is at risk of developing a disease or suffers from a disease resulting from a mutation or a polymorphism in a candidate gene of the present invention. The present invention also provides methods to determine whether an individual is likely to respond positively to an agent acting on a disease or whether an individual is at risk of developing an adverse side effect to an agent acting on a disease.

These methods involve obtaining a nucleic acid sample from the individual and, determining, whether the nucleic acid sample contains at least one allele or at least one biallelic marker haplotype, indicative of a risk of developing the trait or indicative that the individual expresses the trait as a result of possessing a particular candidate gene polymorphism or mutation (trait-causing allele).

Preferably, in such diagnostic methods, a nucleic acid sample is obtained from the individual and this sample is genotyped using methods described above in III. The diagnostics may be based on a single biallelic marker or a on group of biallelic markers.

In each of these methods, a nucleic acid sample is obtained from the test subject and the biallelic marker pattern of one or more of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, and 3735 to 3908 is determined.

In one embodiment, a PCR amplification is conducted on the nucleic acid sample to amplify regions in which polymorphisms associated with a detectable phenotype have been identified. The amplification products are sequenced to determine whether the individual possesses one or more polymorphisms associated with a detectable phenotype. The primers used to generate amplification products may comprise the primers of SEQ ID Nos. 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. Alternatively, the nucleic acid sample is subjected to microsequencing reactions as described above to determine whether the individual possesses one or more polymorphisms associated with a detectable phenotype resulting from a mutation or a polymorphism in a candidate gene. In another embodiment, the nucleic acid sample is contacted with one or more allele specific oligonucleotide probes which, specifically hybridize to one or more candidate gene alleles associated with a detectable phenotype.

These diagnostic methods are extremely valuable as they can, in certain circumstances, be used to initiate preventive treatments or to allow an individual carrying a significant haplotype to foresee warning signs such as minor symptoms. In diseases in which attacks may be extremely violent and sometimes fatal if not treated on time, such as disease, the knowledge of a potential predisposition, even if this predisposition is not absolute, might contribute in a very significant manner to treatment efficacy. Similarly, a diagnosed

predisposition to a potential side effect could immediately direct the physician toward a treatment for which such side effects have not been observed during clinical trials.

Diagnostics, which analyze and predict response to a drug or side effects to a drug, may be used to determine whether an individual should be treated with a particular drug. For example, if the diagnostic indicates a likelihood that an individual will respond positively to treatment with a particular drug, the drug may be administered to the individual. Conversely, if the diagnostic indicates that an individual is likely to respond negatively to treatment with a particular drug, an alternative course of treatment may be prescribed. A negative response may be defined as either the absence of an efficacious response or the presence of toxic side effects.

Clinical drug trials represent another application for the markers of the present invention. One or more markers indicative of response to an agent acting on a disease or to side effects to an agent acting on a disease may be identified using the methods described above. Thereafter, potential participants in clinical trials of such an agent may be screened to identify those individuals most likely to respond favorably to the drug and exclude those likely to experience side effects. In that way, the effectiveness of drug treatment may be measured in individuals who respond positively to the drug, without lowering the measurement as a result of the inclusion of individuals who are unlikely to respond positively in the study and without risking undesirable safety problems.

VI. Computer-Related Embodiments

In some embodiments of the present invention a computer to based system may support the on-line coordination between the identification of biallelic markers and the corresponding analysis of their frequency in the different groups.

As used herein the term "nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773" encompasses the nucleotide sequences of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773, fragments of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773, nucleotide sequences homologous to SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 or homologous to fragments of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773, and sequences complementary to all of the preceding sequences. As used

herein the term "nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773" further encompasses the nucleotide sequences comprising, consisting essentially of, or consisting of any one of the following:

a) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 1 to 2260 or the complements thereof;

b) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 1 to 2260 or the complements thereof, further comprising the 1ST allele of the polymorphic base of the respective SEQ ID number;

c) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 1 to 2260 or the complements thereof, further comprising the 2ND allele of the polymorphic base of the respective SEQ ID number;

d) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 2261 to 3734 or the complements thereof;

e) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 2261 to 3734 or the complements thereof, further comprising the 1ST allele of the polymorphic base of the respective SEQ ID number;

f) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 2261 to 3734 or the complements thereof, further comprising the 2ND allele of the polymorphic base of the respective SEQ ID number;

g) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3735 to 3908 or the complements thereof;

h) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 r 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3735 to 3908 or the complements thereof, further comprising the 1ST allele of the polymorphic base of the respective SEQ ID number;

i) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3735 to 3908 or the complements thereof, further comprising the 2ND allele of the polymorphic base of the respective SEQ ID number; and

j) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, or 21 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 or the complements thereof.

The "nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773" further encompass nucleotide sequences homologous to:

a) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 1 to 2260 or the complements thereof;

b) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 1 to 2260 or the complements thereof, further comprising the 1ST allele of the polymorphic base of the respective SEQ ID number;

c) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 1 to 2260 or the complements thereof, further comprising the 2ND allele of the polymorphic base of the respective SEQ ID number;

d) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 2261 to 3734 or the complements thereof;

e) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 2261 to 3734 or the complements thereof, further comprising the 1ST allele of the polymorphic base of the respective SEQ ID number;

f) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 2261 to 3734 or the complements thereof, further comprising the 2ND allele of the polymorphic base of the respective SEQ ID number;

g) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3735 to 3908 or the complements thereof;

h) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3735 to 3908 or the complements thereof, further comprising the 1ST allele of the polymorphic base of the respective SEQ ID number;

i) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, 22, 23, 24, 25, 30, 35, 43, 44, 45, 46 or 47 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3735 to 3908 or the complements thereof, further comprising the 2ND allele of the polymorphic base of the respective SEQ ID number; and

j) a contiguous span of at least 8, 10, 12, 15, 18, 19, 20, or 21 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of any of SEQ ID Nos. 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 or the complements thereof.

Homologous sequences refer to a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 75% homology to these contiguous spans. Homology may be determined using any method described herein, including BLAST2N with the default parameters or with any modified parameters. Homologous sequences also may include RNA sequences in which uridines replace the thymines in the nucleic acid codes of the invention. It will be appreciated that the nucleic acid codes of the invention can be represented in the traditional single character format (See the inside back cover of Stryer, Lubert. *Biochemistry*, 3rd edition. W. H Freeman &

Co., New York.) or in any other format or code which records the identity of the nucleotides in a sequence.

It should be noted that the nucleic acid codes of the invention further encompass all of the polynucleotides disclosed, described or claimed in the present application. Moveover, the present invention specifically contemplates computer readable media and computer systems wherein such codes are stored individually or in any combination.

It will be appreciated by those skilled in the art that the nucleic acid codes of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 can be stored, recorded, and manipulated on any medium which can be read and accessed by a computer. As used herein, the words "recorded" and "stored" refer to a process for storing information on a computer medium. A skilled artisan can readily adopt any of the presently known methods for recording information on a computer readable medium to generate embodiments comprising one or more of the nucleic acid codes of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. A particularly preferred embodiment of the present invention is a computer readable medium having recorded thereon at least 2, 5, 10, 15, 20, 25, 30, 50, 100, 200, 500, 1000, 2000, or 5000 nucleic acid codes of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773.

Computer readable media include magnetically readable media, optically readable media, electronically readable media and magnetic/optical media. For example, the computer readable media may be a hard disk, a floppy disk, a magnetic tape, CD-ROM, Digital Versatile Disk (DVD), Random Access Memory (RAM), or Read Only Memory (ROM) as well as other types of other media known to those skilled in the art.

Embodiments of the present invention include systems, particularly computer systems which store and manipulate the sequence information described herein. One example of a computer system 100 is illustrated in block diagram form in Figure 14. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to analyze the nucleotide sequences of the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. In one embodiment, the computer system 100 is a Sun Enterprise 1000 server (Sun Microsystems, Palo Alto, CA). The computer system 100 preferably includes a processor for processing, accessing and manipulating the sequence data. The processor 105 can be any well-known type of central

processing unit, such as the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq or International Business Machines.

Preferably, the computer system 100 is a general purpose system that comprises the processor 105 and one or more internal data storage components 110 for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

In one particular embodiment, the computer system 100 includes a processor 105 connected to a bus which is connected to a main memory 115 (preferably implemented as RAM) and one or more internal data storage devices 110, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system 100 further includes one or more data retrieving device 118 for reading the data stored on the internal data storage devices 110.

The data retrieving device 118 may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, etc. In some embodiments, the internal data storage device 110 is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system 100 may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

The computer system 100 includes a display 120 which is used to display output to a computer user. It should also be noted that the computer system 100 can be linked to other computer systems 125a-c in a network or wide area network to provide centralized access to the computer system 100.

Software for accessing and processing the nucleotide sequences of the nucleic acid codes of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 (such as search tools, compare tools, and modeling tools etc.) may reside in main memory 115 during execution.

In some embodiments, the computer system 100 may further comprise a sequence comparer for comparing the above-described nucleic acid codes of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 stored on a computer readable medium to reference nucleotide or polypeptide sequences stored n a computer readable medium. A "sequence comparer" refers to one or more programs which are implemented on the computer system 100 to compare a nucleotide sequence with other

nucleotide sequences and/or compounds stored within the data storage means. For example, the sequence comparer may compare the nucleotide sequences of the nucleic acid codes of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 stored on a computer readable medium to reference sequences stored on a computer readable medium to identify homologies or structural motifs. The various sequence comparer programs identified elsewhere in this patent specification are particularly contemplated for use in this aspect of the invention.

Figure 15 is a flow diagram illustrating one embodiment of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system 100, or a public database such as GENBANK that is available through the Internet.

The process 200 begins at a start state 201 and then moves to a state 202 wherein the new sequence to be compared is stored to a memory in a computer system 100. As discussed above, the memory could be any type of memory, including RAM or an internal storage device.

The process 200 then moves to a state 204 wherein a database of sequences is opened for analysis and comparison. The process 200 then moves to a state 206 wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state 210 to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the homology level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system.

Once a comparison of the two sequences has been performed at the state 210, a determination is made at a decision state 210 whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the homology parameters entered by the user will be marked as "same" in the process 200.

If a determination is made that the two sequences are the same, the process 200 moves to a state 214 wherein the name of the sequence from the database is displayed to the user. This state notifies the user that the sequence with the displayed name fulfills the homology constraints that were entered. Once the name of the stored sequence is displayed to the user, the process 200 moves to a decision state 218 wherein a determination is made whether more sequences exist in

the database. If no more sequences exist in the database, then the process 200 terminates at an end state 220. However, if more sequences do exist in the database, then the process 200 moves to a state 224 wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database.

It should be noted that if a determination had been made at the decision state 212 that the sequences were not homologous, then the process 200 would move immediately to the decision state 218 in order to determine if any other sequences were available in the database for comparison.

Accordingly, one aspect of the present invention is a computer system comprising a processor, a data storage device having stored thereon a nucleic acid code of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773, a data storage device having retrievably stored thereon reference nucleotide sequences or polypeptide sequences to be compared to the nucleic acid code of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 and a sequence comparer for conducting the comparison. The sequence comparer may indicate a homology level between the sequences compared or identify structural motifs in the above described nucleic acid code of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 or it may identify structural motifs in sequences which are compared to these nucleic acid codes and polypeptide codes. In some embodiments, the data storage device may have stored thereon the sequences of at least 2, 5, 10, 15, 20, 25, 30, 50, 100, 200, 500, 1000, 2000, or 5000 of the nucleic acid codes of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773.

Another aspect of the present invention is a method for determining the level of homology between a nucleic acid code of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 and a reference nucleotide sequence, comprising the steps of reading the nucleic acid code and the reference nucleotide sequence through the use of a computer program which determines homology levels and determining homology between the nucleic acid code and the reference nucleotide sequence with the computer program. The computer program may be any of a number of c mputer programs for determining homology levels, including those specifically enumerated herein, including

BLAST2N with the default parameters or with any modified parameters. The method may be implemented using the computer systems described above. The method may also be performed by reading at least 2, 5, 10, 15, 20, 25, 30, 50, 100, 200, 500, 1000, 2000, or 5000 of the above described nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 through use of the computer program and determining homology between the nucleic acid codes and reference nucleotide sequences .

Figure 16 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous. The process 250 begins at a start state 252 and then moves to a state 254 wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state 256. The process 250 then moves to a state 260 wherein the first character in the first sequence is read and then to a state 262 wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it should be in the single letter amino acid code so that the first and sequence sequences can be easily compared.

A determination is then made at a decision state 264 whether the two characters are the same. If they are the same, then the process 250 moves to a state 268 wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters are the same. If they are, then the process 250 continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process 250 moves to a decision state 274 to determine whether there are any more characters either sequence to read.

If there aren't any more characters to read, then the process 250 moves to a state 276 wherein the level of homology between the first and second sequences is displayed to the user. The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the homology level would be 100%.

Alternatively, the computer program may be a computer program which compares the nucleotide sequences of the nucleic acid codes of the present invention, to reference nucleotide sequences in order to determine whether the nucleic acid code of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 differs from a reference nucleic acid sequence at one or more positions. Optionally such a program records the length

and identity of inserted, deleted or substituted nucleotides with respect to the sequence of either the reference polynucleotide or the nucleic acid code of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 t 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. In one embodiment, the computer program may be a program which determines whether the nucleotide sequences of the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 contain a biallelic marker or single nucleotide polymorphism (SNP) with respect to a reference nucleotide sequence. This single nucleotide polymorphism may comprise a single base substitution, insertion, or deletion, while this biallelic marker may comprise about one to ten consecutive bases substituted, inserted or deleted.

Accordingly, another aspect of the present invention is a method for determining whether a nucleic acid code of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 differs at one or more nucleotides from a reference nucleotide sequence comprising the steps of reading the nucleic acid code and the reference nucleotide sequence through use of a computer program which identifies differences between nucleic acid sequences and identifying differences between the nucleic acid code and the reference nucleotide sequence with the computer program. In some embodiments, the computer program is a program which identifies single nucleotide polymorphisms. The method may be implemented by the computer systems described above and the method illustrated in Figure 16. The method may also be performed by reading at least 2, 5, 10, 15, 20, 25, 30, 50, 100, 200, 500, 1000, 2000, or 5000 of the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 and the reference nucleotide sequences through the use of the computer program and identifying differences between the nucleic acid codes and the reference nucleotide sequences with the computer program.

In other embodiments the computer based system may further comprise an identifier for identifying features within the nucleotide sequences of the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773.

An "identifier" refers to one or more programs which identifies certain features within the above-described nucleotide sequences of the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. In one

embodiment, the identifier may comprise a program which identifies an open reading frame in the cDNAs codes f SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773.

Figure 17 is a flow diagram illustrating one embodiment of an identifier process 300 for detecting the presence of a feature in a sequence. The process 300 begins at a start state 302 and then moves to a state 304 wherein a first sequence that is to be checked for features is stored to a memory 115 in the computer system 100. The process 300 then moves to a state 306 wherein a database of sequence features is opened. Such a database would include a list of each feature's attributes along with the name of the feature. For example, a feature name could be "Initiation Codon" and the attribute would be "ATG". Another example would be the feature name "TAATAA Box" and the feature attribute would be "TAATAA". An example of such a database is produced by the University of Wisconsin Genetics Computer Group (www.gcg.com).

Once the database of features is opened at the state 306, the process 300 moves to a state 308 wherein the first feature is read from the database. A comparison of the attribute of the first feature with the first sequence is then made at a state 310. A determination is then made at a decision state 316 whether the attribute of the feature was found in the first sequence. If the attribute was found, then the process 300 moves to a state 318 wherein the name of the found feature is displayed to the user.

The process 300 then moves to a decision state 320 wherein a determination is made whether move features exist in the database. If no more features do exist, then the process 300 terminates at an end state 324. However, if more features do exist in the database, then the process 300 reads the next sequence feature at a state 326 and loops back to the state 310 wherein the attribute of the next feature is compared against the first sequence.

It should be noted, that if the feature attribute is not found in the first sequence at the decision state 316, the process 300 moves directly to the decision state 320 in order to determine if any more features exist in the database.

Accordingly, another aspect of the present invention is a method of identifying a feature within the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 comprising reading the nucleic acid code(s) through the use of a computer program which identifies features therein and identifying features within the nucleic acid code(s) with the computer program. In one embodiment, computer program comprises a computer program which identifies open reading frames. The method may be performed by reading a single sequence or at least 2, 5, 10, 15, 20, 25, 30, 50,

100, 200, 500, 1000, 2000, or 5000 of the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 through the use of the computer program and identifying features within the nucleic acid codes with the computer program.

The nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 may be stored and manipulated in a variety of data processor programs in a variety of formats. For example, the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 may be stored as text in a word processing file, such as MicrosoftWORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE. In addition, many computer programs and databases may be used as sequence comparers, identifiers, or sources of reference nucleotide sequences to be compared to the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. The following list is intended not to limit the invention but to provide guidance to programs and databases which are useful with the nucleic acid codes of SEQ ID NOs. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908, 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773.

The programs and databases which may be used include, but are not limited to: MacPattern (EMBL), DiscoveryBase (Molecular Applications Group), GeneMine (Molecular Applications Group), Look (Molecular Applications Group), MacLook (Molecular Applications Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul et al, *J. Mol. Biol.* 215: 403 (1990)), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, 85: 2444 (1988)), FASTDB (Brutlag et al. Comp. App. Biosci. 6:237-245, 1990), Catalyst (Molecular Simulations Inc.), Catalyst/SHAPE (Molecular Simulations Inc.), Cerius^2.DBAccess (Molecular Simulations Inc.), HypoGen (Molecular Simulations Inc.), Insight II, (Molecular Simulations Inc.), Discover (Molecular Simulations Inc.), CHARMm (Molecular Simulations Inc.), Felix (Molecular Simulations Inc.), DelPhi, (Molecular Simulations Inc.), QuanteMM, (Molecular Simulations Inc.), Homology (Molecular Simulations Inc.), Modeler (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), Quanta/Protein Design (Molecular Simulations Inc.), WebLab (Molecular Simulations Inc.), WebLab Diversity Explorer (Molecular Simulations Inc.), Gene Explorer (Molecular Simulations Inc.), SeqFold (Molecular Simulations Inc.), the MDL

Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, Derwents's World Drug Index database, the BioByteMasterFile database, the Genbank database, and the Genseqn database. Many other programs and data bases would be apparent to one of skill in the art given the present disclosure.

Motifs which may be detected using the above programs include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and beta sheets, signal sequences encoding signal peptides which direct the secretion of the encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

It should be noted that the nucleic acid codes of the invention further encompass all of the polynucleotides disclosed, described or claimed in the present application. Moreover, the present invention specifically contemplates the storage of such codes on computer readable media and computer systems individually or in any combination, as well as the use of such codes and combinations in the methods of VI.

VII. Mapping and Maps Comprising the Biallelic Markers of the Invention

The human haploid genome contains an estimated 80,000 to 100,000 or more genes scattered on a 3 x 10^9 base-long double stranded DNA shared among the 24 chromosomes. Each human being is diploid, *i.e.* possesses two haploid genomes, one from paternal origin, the other from maternal origin. The sequence of the human genome varies among individuals in a population About 10^7 sites scattered along the 3 x 10^9 base pairs of DNA are polymorphic, existing in at least two variant forms called alleles. Most of these polymorphic sites are generated by single base substitution mutations and are biallelic. Less than 10^5 polymorphic sites are due to more complex changes and are very often multi-allelic, i.e. exist in more than two allelic forms. At a given polymorphic site, any individual (diploid), can be either homozygous (twice the same allele) or heterozygous (two different alleles). A given polymorphism or rare mutation can be either neutral (no effect on trait), or functional, *i.e.* responsible for a particular genetic trait.

Genetic Maps

The first step towards the identification of genes associated with a detectable trait, such as a disease or any other detectable trait, consists in the localization of genomic regions containing trait-causing genes using genetic mapping methods. The preferred traits contemplated within the present invention relate to fields of therapeutic interest; in particular embodiments, they will be disease traits and/or drug response traits, reflecting drug efficacy or toxicity. Traits can either be "binary", e.g. diabetic vs. non diabetic, or "quantitative", e.g. elevated blood pressure. Individuals affected by a quantitative trait can be classified according to an appropriate scale of trait values, e.g. blood pressure ranges. Each trait value

range can then be analyzed as a binary trait. Patients showing a trait value within one such range will be studied in comparison with patients showing a trait value outside of this range. In such a case, genetic analysis methods will be applied to subpopulations of individuals showing trait values within defined ranges.

Genetic mapping involves the analysis of the segregation of polymorphic loci in trait positive and trait-negative populations. Polymorphic loci constitute a small fraction of the human genome (less than 1%), compared to the vast majority of human genomic DNA which is identical in sequence among the chromosomes of different individuals. Among all existing human polymorphic loci, genetic markers can be defined as genome-derived polynucleotides which are sufficiently polymorphic to allow a reasonable probability that a randomly selected person will be heterozygous, and thus informative for genetic analysis by methods such as linkage analysis or association studies.

A genetic map consists of a collection of polymorphic markers which have been positioned on the human chromosomes. Genetic maps may be combined with physical maps, collections of ordered overlapping fragments of genomic DNA whose arrangement along the human chromosomes is known. The optimal genetic map should possess the following characteristics:

- the density of the genetic markers scattered along the genome should be sufficient to allow the identification and localization of any trait-related polymorphism,
- each marker should have an adequate level of heterozygosity, so as to be informative in a large percentage of different meioses,
- all markers should be easily typed on a routine basis, at a reasonable expense, and in a reasonable amount of time,
- the entire set of markers per chromosome should be ordered in a highly reliable fashion.

However, while the above maps are optimal, it will be appreciated that the maps of the present invention may be used in the individual marker and haplotype association analyses described below without the necessity of determining the order of biallelic markers derived from a single BAC with respect to one another.

Construction of a Physical Map

The first step in constructing a high density genetic map of biallelic markers is the construction of a physical map. Physical maps consist of ordered, overlapping cloned fragments of genomic DNA covering a portion of the genome, preferably covering one or all chromosomes. Obtaining a physical map of the genome entails constructing and rdering a genomic DNA library. For an example of a complete explanation of the construction of a physical map from a BAC library see related PCT Application No. PCT/IB98/00193 filed July

19, 1998. The methods disclosed therein can be used to generate larger more complete sets of markers and entire maps of the human genome comprising the map-relate biallelic markers of the invention.

Biallelic Markers

It will be appreciated that the ordered DNA fragments containing these groups of biallelic markers need not completely cover the genomic regions of these lengths but may instead be incomplete contigs having one or more gaps therein. As discussed in further detail below, biallelic markers may be used in single maker and haplotype association analyses regardless of the completeness of the corresponding physical contig harboring them.

Using the procedures above, 3908 biallelic markers, each having two alleles, were identified using sequences obtained from BACs which had been localized on the genome. In some cases, markers were identified using pooled BACs and thereafter reassigned to individual BACs using STS screening procedures such as those described in Examples 1 and 2. The sequences of these biallelic markers are provided in the accompanying Sequence Listing as SEQ ID Nos. 1 to 3908. Although the sequences of SEQ ID Nos. 1 to 3908 will be used as exemplary markers throughout the present application, these markers are not limited to markers having the exact flanking sequences surrounding the polymorphic bases which are enumerated in SEQ ID Nos. 1 to 3908. Rather, it will be appreciated that the flanking sequences surrounding the polymorphic bases of SEQ ID Nos. 1 to 3908 may be lengthened or shortened to any extent compatible with their intended use and the present invention specifically contemplates such sequences. The sequences of these biallelic markers may be used to construct genomic maps as well as in the gene identification and diagnostic techniques described herein. It will be appreciated that the biallelic markers referred to herein may be of any length compatible with their intended use provided that the markers include the polymorphic base, and the present invention specifically contemplates such sequences.

Some of the markers of SEQ ID Nos: 1 to 3908 as well as related amplification and microsequencing primers were disclosed in the instant priority documents. However, some of the earlier described amplification primers and microsequencing primers did not have the precise sequence lengths disclosed in the instant application. It will be appreciated that either length of primers may be used in the methods disclosed in the present application.

In addition, the internal identification numbers used to identify the biallelic markers disclosed in U.S. Provisional Patent Application Serial No. 60/082,614 filed April 21, 1998 have been revised to include additional numbers on the end. For example, the marker formerly given the internal identification number 99-1091 was given the revised internal identification number 99-1091-446. Therefore, it will be appreciated that shortened

identificati n numbers and extended identification numbers which overlap one another refer to the same markers.

Ordering of biallelic markers

Biallelic markers can be ordered to determine their positions along chromosomes, preferably subchromosomal regions, by methods known in the art as well as those disclosed in PCT Application No. PCT/IB98/00193 filed July 19, 1998, and U.S. Provisional Patent Application Serial No. 60/082,614 filed April 21, 1998.

The positions of the biallelic markers along chromosomes may be determined using a variety of methodologies. In one approach, radiation hybrid mapping is used. Radiation hybrid (RH) mapping is a somatic cell genetic approach that can be used for high resolution mapping of the human genome. In this approach, cell lines containing one or more human chromosomes are lethally irradiated, breaking each chromosome into fragments whose size depends on the radiation dose. These fragments are rescued by fusion with cultured rodent cells, yielding subclones containing different portions of the human genome. This technique is described by Benham et al. (*Genomics* 4:509-517, 1989) and Cox et al., (*Science* 250:245-250, 1990). The random and independent nature of the subclones permits efficient mapping of any human genome marker. Human DNA isolated from a panel of 80-100 cell lines provides a mapping reagent for ordering biallelic markers. In this approach, the frequency of breakage between markers is used to measure distance, allowing construction of fine resolution maps as has been done for ESTs (Schuler et al., *Science* 274:540-546, 1996).

RH mapping has been used to generate a high-resolution whole genome radiation hybrid map of human chromosome 17q22-q25.3 across the genes for growth hormone (GH) and thymidine kinase (TK) (Foster et al., *Genomics* 33:185-192, 1996), the region surrounding the Gorlin syndrome gene (Obermayr et al., *Eur. J. Hum. Genet.* 4:242-245, 1996), 60 loci covering the entire short arm of chromosome 12 (Raeymaekers et al., *Genomics* 29:170-178, 1995), the region of human chromosome 22 containing the neurofibromatosis type 2 locus (Frazer et al., *Genomics* 14:574-584, 1992) and 13 loci on the long arm of chromosome 5 (Warrington et al., *Genomics* 11:701-708, 1991).

Alternatively, PCR based techniques and human-rodent somatic cell hybrids may be used to determine the positions of the biallelic markers on the chromosomes. In such approaches, oligonucleotide primer pairs which are capable of generating amplification products containing the polymorphic bases of the biallelic markers are designed. Preferably, the oligonucleotide primers are 18-23 bp in length and are designed for PCR amplification. The creation of PCR primers from known sequences is well known to those with skill in the art. For a review of PCR technology see Erlich, H.A., *PCR Technology: Principles and Applications for DNA Amplification*. 1992. W.H. Freeman and Co., New York.

The primers are used in polymerase chain reactions (PCR) to amplify templates from total human genomic DNA. PCR conditions are as follows: 60 ng of genomic DNA is used as a template for PCR with 80 ng of each oligonucleotide primer, 0.6 unit of Taq polymerase, and 1 mCu of a ^{32}P-labeled deoxycytidine triphosphate. The PCR is performed in a microplate thermocycler (Techne) under the following conditions: 30 cycles of 94°C, 1.4 min; 55°C, 2 min; and 72°C, 2 min; with a final extension at 72°C for 10 min. The amplified products are analyzed on a 6% polyacrylamide sequencing gel and visualized by autoradiography. If the length of the resulting PCR product is identical to the length expected for an amplification product containing the polymorphic base of the biallelic marker, then the PCR reaction is repeated with DNA templates from two panels of human-rodent somatic cell hybrids, BIOS PCRable DNA (BIOS Corporation) and NIGMS Human-Rodent Somatic Cell Hybrid Mapping Panel Number 1 (NIGMS, Camden, NJ).

PCR is used to screen a series of somatic cell hybrid cell lines containing defined sets of human chromosomes for the presence of a given biallelic marker. DNA is isolated from the somatic hybrids and used as starting templates for PCR reactions using the primer pairs from the biallelic marker. Only those somatic cell hybrids with chromosomes containing the human sequence corresponding to the biallelic marker will yield an amplified fragment. The biallelic markers are assigned to a chromosome by analysis of the segregation pattern of PCR products from the somatic hybrid DNA templates. The single human chromosome present in all cell hybrids that give rise to an amplified fragment is the chromosome containing that biallelic marker. For a review of techniques and analysis of results from somatic cell gene mapping experiments. (See Ledbetter et al., *Genomics* 6:475-481 (1990).)

Example 2 describes a preferred method for positioning of biallelic markers on clones, such as BAC clones, obtained from genomic DNA libraries. Using such procedures, a number of BAC clones carrying selected biallelic markers can be isolated. The position of these BAC clones on the human genome can be defined by performing STS screening as described in Example 1. Preferably, to decrease the number of STSs to be tested, each BAC can be localized on chromosomal or subchromosomal regions by procedures such as those described in Examples 3 and 4. This localization will allow the selection of a subset of STSs corresponding to the identified chromosomal or subchromosomal region. Testing each BAC with such a subset of STSs and taking account of the position and order of the STSs along the genome will allow a refined positioning of the corresponding biallelic marker along the genome.

In other embodiments, if the DNA library used to isolate BAC inserts or any type of genomic DNA fragments harboring the selected biallelic markers already constitute a physical map of the genome or any portion thereof, using the known order of the DNA fragments will

allow the order of the biallelic markers to be established.

As discussed above, it will be appreciated that markers carried by the same fragment of genomic DNA, such as the insert in a BAC clone, need not necessarily be ordered with respect to one another within the genomic fragment to conduct single point or haplotype association analyses. However, in other embodiments of the present maps, the order of biallelic markers carried by the same fragment of genomic DNA may be determined.

The positions of the biallelic markers used to construct the maps of the present invention, including the map-related biallelic markers of the invention, may be assigned to subchromosomal locations using Fluorescence In Situ Hybridization (FISH) (Cherif et al., *Proc. Natl. Acad. Sci. U.S.A.*, 87:6639-6643 (1990)). FISH analysis is described in Example 3.

The ordering analyses may be conducted to generate an integrated genome wide genetic map comprising about 20,000, 40,000, 60,000, 80,000, 100,000, 120,000 biallelic markers with a roughly consistent number of biallelic marker per BAC. In some embodiments, the map includes one or more markers selected from the group consisting of the sequences of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto.

Alternatively, maps having the above-specified average numbers of biallelic markers per BAC which comprise smaller portions of the genome, such as a set of chromosomes, a single chromosome, a particular subchromosomal region, or any other desired portion of the genome, may also be constructed using the procedures provided herein.

In some embodiments, the biallelic markers in the map are separated from one another by an average distance of 10-200kb, 15-150kb, 20-100kb, 100-150kb, 50-100kb, or 25-50kb. Maps having the above-specified intermarker distances which comprise smaller portions of the genome, such as a set of chromosomes, a single chromosome, a particular subchromosomal region, or any other desired portion of the genome, may also be constructed using the procedures provided herein.

Figure 2, showing the results of computer simulations of the distribution of inter-marker spacing on a randomly distributed set of biallelic markers, indicates the percentage of biallelic markers which will be spaced a given distance apart for a given number of markers/BAC in the genomic map (assuming 20,000 BACs constituting a minimally overlapping array covering the entire genome are evaluated). One hundred iterations were performed for each simulation (20,000 marker map, 40,000 marker map, 60,000 marker map, 120,000 marker map).

As illustrated in Figure 2a, 98% f inter-marker distances will be lower than 150kb provided 60,000 evenly distributed markers are generated (3 per BAC); 90% of inter-marker distances will be lower than 150kb provided 40,000 evenly distributed markers are generated

(2 per BAC); and 50% of inter-marker distances will be lower than 150kb provided 20,000 evenly distributed markers are generated (1 per BAC).

As illustrated in Figure 2b, 98% of inter-marker distances will be lower than 80kb provided 120,000 evenly distributed markers are generated (6 per BAC); 80% of inter-marker distances will be lower than 80kb provided 60,000 evenly distributed markers are generated (3 per BAC); and 15% of inter-marker distances will be lower than 80kb provided 20,000 evenly distributed markers are generated (1 per BAC).

As already mentioned, high density biallelic marker maps allow association studies to be performed to identify genes involved in complex traits.

Linkage Disequilibrium

The present invention then also concerns biallelic markers in linkage disequilibrium with the specific biallelic markers described above and which are expected to present similar characteristics in terms of their respective association with a given trait. In a preferred embodiment, the present invention concerns the biallelic markers that are in linkage disequilibrium with the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto.

LD among a set of biallelic markers having an adequate heterozygosity rate can be determined by genotyping between 50 and 1000 unrelated individuals, preferably between 75 and 200, more preferably around 100. Genotyping a biallelic marker consists of determining the specific allele carried by an individual at the given polymorphic base of the biallelic marker. Genotyping can be performed using similar methods as those described above for the generation of the biallelic markers, or using other genotyping methods such as those further described below.

Genome-wide linkage disequilibrium mapping aims at identifying, for any trait-causing allele being searched, at least one biallelic marker in linkage disequilibrium with said trait-causing allele. Preferably, in order to enhance the power of linkage disequilibrium maps, in some embodiments, the biallelic markers therein have average inter-marker distances of 150kb or less, 75 kb or less, or 50 kb or less, 30kb or less, or 25kb or less to accommodate the fact that, in some regions of the genome, the detection of linkage disequilibrium requires lower inter-marker distances.

The present invention provides methods to generate biallelic marker maps with average inter-marker distances of 150kb or less. In some embodiments, the mean distance between biallelic markers constituting the high density map will be less than 75kb, preferably less than 50kb. Further preferred maps according to the present invention contain markers that are less than 37.5kb apart. In highly preferred embodiments, the average inter-marker spacing

for the biallelic markers constituting very high density maps is less than 30kb, most preferably less than 25kb.

Genetic maps containing biallelic markers (including the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto) may be used to identify and isolate genes associated with detectable traits. The use of the genetic maps of the present invention is described in more detail below.

VIII. Use of High Density Biallelic Marker Maps to Identify Genes Associated with Detectable Traits

One embodiment of the present invention comprises methods for identifying and isolating genes associated with a detectable trait using the biallelic marker maps of the present invention.

In the past, the identification of genes linked with detectable traits has relied on a statistical approach called linkage analysis. Linkage analysis is based upon establishing a correlation between the transmission of genetic markers and that of a specific trait throughout generations within a family. In this approach, all members of a series of affected families are genotyped with a few hundred markers, typically microsatellite markers, which are distributed at an average density of one every 10 Mb. By comparing genotypes in all family members, one can attribute sets of alleles to parental haploid genomes (haplotyping or phase determination). The origin of recombined fragments is then determined in the offspring of all families. Those that co-segregate with the trait are tracked. After pooling data from all families, statistical methods are used to determine the likelihood that the marker and the trait are segregating independently in all families. As a result of the statistical analysis, one or several regions having a high probability of harboring a gene linked to the trait are selected as candidates for further analysis. The result of linkage analysis is considered as significant (i.e. there is a high probability that the region contains a gene involved in a detectable trait) when the chance of independent segregation of the marker and the trait is lower than 1 in 1000 (expressed as a LOD score > 3). Generally, the length of the candidate region identified using linkage analysis is between 2 and 20Mb.

Once a candidate region is identified as described above, analysis of recombinant individuals using additional markers allows further delineation of the candidate linked region.

Linkage analysis studies have generally relied on the use of a maximum of 5,000 microsatellite markers, thus limiting the maximum theoretical attainable resolution of linkage analysis to ca. 600 kb on average.

Linkage analysis has been successfully applied to map simple genetic traits that show clear Mendelian inheritance patterns and which have a high penetrance (penetrance is the ratio between the number of trait-positive carriers of allele *a* and the total number of *a* carriers in

the population). About 100 pathological trait-causing genes were discovered using linkage analysis over the last 10 years. In most of these cases, the majority of affected individuals had affected relatives and the detectable trait was rare in the general population (frequencies less than 0.1%). In about 10 cases, such as Alzheimer's Disease, breast cancer, and Type II diabetes, the detectable trait was more common but the allele associated with the detectable trait was rare in the affected population. Thus, the alleles associated with these traits were not responsible for the trait in all sporadic cases.

Linkage analysis suffers from a variety of drawbacks. First, linkage analysis is limited by its reliance on the choice of a genetic model suitable for each studied trait. Furthermore, as already mentioned, the resolution attainable using linkage analysis is limited, and complementary studies are required to refine the analysis of the typical 2Mb to 20Mb regions initially identified through linkage analysis.

In addition, linkage analysis approaches have proven difficult when applied to complex genetic traits, such as those due to the combined action of multiple genes and/or environmental factors. In such cases, too large an effort and cost are needed to recruit the adequate number of affected families required for applying linkage analysis to these situations, as recently discussed by Risch, N. and Merikangas, K. (*Science* 273:1516-1517 (1996)).

Finally, linkage analysis cannot be applied to the study of traits for which no large informative families are available. Typically, this will be the case in any attempt to identify trait-causing alleles involved in sporadic cases, such as alleles associated with positive or negative responses to drug treatment.

The present genetic maps and biallelic markers (including the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto) may be used to identify and isolate genes associated with detectable traits using association studies, an approach which does not require the use of affected families and which permits the identification of genes associated with sporadic traits.

Association Studies

As already mentioned, any gene responsible or partly responsible for a given trait will be in linkage disequilibrium with some flanking markers. To map such a gene, specific alleles of these flanking markers which are associated with the gene or genes responsible for the trait are identified. Although the following discussion of techniques for finding the gene or genes associated with a particular trait using linkage disequilibrium mapping, refers to locating a single gene which is responsible for the trait, it will be appreciated that the same techniques may also be used to identify genes which are partially responsible for the trait.

Association studies may be conducted within the general population (as opposed to the linkage analysis techniques discussed above which are limited to studies performed on related individuals in one or several affected families).

Association between a biallelic marker A and a trait T may primarily occur as a result of three possible relationships between the biallelic marker and the trait.

First, allele *a* of biallelic marker A may be directly responsible for trait T (e.g., Apo E ∈ 4 site A and Alzheimer's disease). However, since the majority of the biallelic markers used in genetic mapping studies are selected randomly, they mainly map outside of genes. Thus, the likelihood of allele *a* being a functional mutation directly related to trait T is very low.

Second, an association between a biallelic marker A and a trait T may also occur when the biallelic marker is very closely linked to the trait locus. In other words, an association occurs when allele *a* is in linkage disequilibrium with the trait-causing allele. When the biallelic marker is in close proximity to a gene responsible for the trait, more extensive genetic mapping will ultimately allow a gene to be discovered near the marker locus which carries mutations in people with trait T (i.e. the gene responsible for the trait or one of the genes responsible for the trait). As will be further exemplified below, using a group of biallelic markers which are in close proximity to the gene responsible for the trait the location of the causal gene can be deduced from the profile of the association curve between the biallelic markers and the trait. The causal gene will usually be found in the vicinity of the marker showing the highest association with the trait.

Finally, an association between a biallelic marker and a trait may occur when people with the trait and people without the trait correspond to genetically different subsets of the population who, coincidentally, also differ in the frequency of allele *a* (population stratification). This phenomenon may be avoided by using ethnically matched large heterogeneous samples.

Association studies are particularly suited to the efficient identification of genes that present common polymorphisms, and are involved in multifactorial traits whose frequency is relatively higher than that of diseases with monofactorial inheritance.

Association studies mainly consist of four steps: recruitment of trait-positive (T+) and control populations, preferably trait-negative (T-) populations with well-defined phenotypes, identification of a candidate region suspected of harboring a trait causing gene, identification of said gene among candidate genes in the region, and finally validation of mutation(s) responsible for the trait in said trait causing gene.

In a first step, the trait-positive should be well-defined, preferably the control phenotype is a well-defined trait-negative phenotype as well. In order to perform efficient and

significant association studies such as those described herein, the trait under study should preferably follow a bimodal distribution in the population under study, presenting two clear non-overlapping phenotypes, trait-positive and trait-negative.

Nevertheless, in the absence of such a bimodal distribution (as may in fact be the case for complex genetic traits), any genetic trait may still be analyzed using the association method proposed herein by carefully selecting the individuals to be included in the trait-positive group and preferably the trait-negative phenotypic group as well. The selection procedure ideally involves selecting individuals at opposite ends of the non-bimodal phenotype spectrum of the trait under study, so as to include in these trait-positive and trait-negative populations individuals who clearly represent non-overlapping, preferably extreme phenotypes.

As discussed above, the definition of the inclusion criteria for the trait-positive and control populations is an important aspect of the present invention.

Figure 3 shows, for a series of hypothetical sample sizes, the p-value significance obtained in association studies performed using individual markers from the high-density biallelic map, according to various hypotheses regarding the difference of allelic frequencies between the trait-positive and trait-negative samples. It indicates that, in all cases, samples ranging from 150 to 500 individuals are numerous enough to achieve statistical significance. It will be appreciated that bigger or smaller groups can be used to perform association studies according to the methods of the present invention.

In a second step, a marker/trait association study is performed that compares the genotype frequency of each biallelic marker in the above described trait-positive and trait-negative populations by means of a chi square statistical test (one degree of freedom). In addition to this single marker association analysis, a haplotype association analysis is performed to define the frequency and the type of the ancestral carrier haplotype. Haplotype analysis, by combining the informativeness of a set of biallelic markers increases the power of the association analysis, allowing false positive and/or negative data that may result from the single marker studies to be eliminated.

Genotyping can be performed using any method described in III, including the microsequencing procedure described in Example 8.

If a positive association with a trait is identified using an array of biallelic markers having a high enough density, the causal gene will be physically located in the vicinity of the associated markers, since the markers showing positive association with the trait are in linkage disequilibrium with the trait locus. Regions harboring a gene responsible for a particular trait which are identified through association studies using high density sets of biallelic markers will, on average, be 20 - 40 times shorter in length than those identified by linkage analysis.

Once a positive association is confirmed as described above, a third step consists of completely sequencing the BAC inserts harboring the markers identified in the association analyzes. These BACs are obtained through screening human genomic libraries with the markers probes and/or primers, as described above. Once a candidate region has been sequenced and analyzed, the functional sequences within the candidate region (e.g. exons, splice sites, promoters, and other potential regulatory regions) are scanned for mutations which are responsible for the trait by comparing the sequences of the functional regions in a selected number of trait-positive and trait-negative individuals using appropriate software. Tools for sequence analysis are further described in Example 9.

Finally, candidate mutations are then validated by screening a larger population of trait-positive and trait-negative individuals using genotyping techniques described below. Polymorphisms are confirmed as candidate mutations when the validation population shows association results compatible with those found between the mutation and the trait in the test population.

In practice, in order to define a region bearing a candidate gene, the trait-positive and trait-negative populations are genotyped using an appropriate number of biallelic markers. The markers may include one or more of the markers of SEQ ID Nos: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto.

The markers used to define a region bearing a candidate gene may be distributed at an average density of 1 marker per 10-200 kb. Preferably, the markers used to define a region bearing a candidate gene are distributed at an average density of 1 marker every 15-150 kb. In further preferred embodiments, the markers used to define a region bearing a candidate gene are distributed at an average density of 1 marker every 20-100kb. In yet another preferred embodiment, the markers used to define a region bearing a candidate gene are distributed at an average density of 1 marker every 100 to 150kb. In a further highly preferred embodiment, the markers used to define a region bearing a candidate gene are distributed at an average density of 1 marker every 50 to 100kb. In yet another embodiment, the biallelic markers used to define a region bearing a candidate gene are distributed at an average density of 1 marker every 25-50 kilobases. As mentioned above, in order to enhance the power of linkage disequilibrium based maps, in a preferred embodiment, the marker density of the map will be adapted to take the linkage disequilibrium distribution in the genomic region of interest into account.

In some embodiments, the initial identification of a candidate genomic region harboring a gene associated with a detectable phenotype may be conducted using a preliminary map containing a few thousand biallelic markers. Thereafter, the genomic region harboring the gene responsible for the detectable trait may be better delineated using a map

containing a larger number of biallelic markers. Furthermore, the genomic region harboring the gene responsible for the detectable trait may be further delineated using a high density map of biallelic markers. Finally, the gene associated with the detectable trait may be identified and isolated using a very high density biallelic marker map.

Example 6 describes a procedure for identifying a candidate region harboring a gene associated with a detectable trait and provides simulated results for this procedure. It will be appreciated that although Example 6 compares the results of simulated analyzes using markers derived from maps having 3,000, 20,000, and 60,000 markers, the number of markers contained in the map is not restricted to these exemplary figures. Rather, Example 6 exemplifies the increasing refinement of the candidate region with increasing marker density. As increasing numbers of markers are used in the analysis, points in the association analysis become broad peaks. The gene associated with the detectable trait under investigation will lie within or near the region under the peak.

The statistical power of linkage disequilibrium mapping using a high density marker map is also reinforced by complementing the single point association analysis described above with a multi-marker association analysis of haplotype analysis described in IV. To improve the statistical power of the individual marker association analyses conducted as described above using maps of increasing marker densities, haplotype studies can be performed using groups of markers located in proximity to one another within regions of the genome. For example, using the methods described above in which the association of an individual marker with a detectable phenotype was analyzed using maps of 3,000 markers, 20,000 markers, and 60,000 markers, a series of haplotype studies can be performed using groups of contiguous markers from such maps or from maps having higher marker densities.

In a preferred embodiment, a series of successive haplotype studies including groups of markers spanning regions of more than 1 Mb may be performed. In some embodiments, the biallelic markers included in each of these groups may be located within a genomic region spanning less than 1kb, from 1 to 5kb, from 5 to 10kb, from 10 to 25kb, from 25 to 50kb, from 50 to 150kb, from 150 to 250kb, from 250 to 500kb, from 500kb to 1Mb, or more than 1Mb. Preferably, the genomic regions containing the groups of biallelic markers used in the successive haplotype analyses are overlapping. It will be appreciated that the groups of biallelic markers need not completely cover the genomic regions of the above-specified lengths but may instead be obtained from incomplete contigs having one or more gaps therein. As discussed in further detail below, biallelic markers may be used in single point and haplotype association analyses regardless of the completeness of the corresponding physical contig harboring them.

Genome-wide mapping using association studies with dense enough arrays of markers

permit a case-by-case best estimate of p-value significance thresholds. Given a test population comprising two ethnically matched trait-positive and control groups of about 50 to about 500 individuals or more, conducting the above described association studies will allow a p-value "cut-off" to be established by, for example, analyzing significant numbers of allele frequency differences or, in some cases where appropriate, running computer simulations or control studies as described in Examples 6, 15, and 26.

For a p-value above the threshold, a corresponding association between the trait and a studied marker will be deemed not significant, while for a p-value below such a threshold, said association will be deemed significant. If the p-value is significant, the genomic region around the marker will be further scrutinized for a trait-causing gene.

It is preferred that p-value significance thresholds be assessed for each case/control population comparison. Both the genetic distance between sampled population- "stratification"-and the dispersion due to random selection of samples may indeed influence the p-value significance thresholds.

It will be appreciated that the above approaches may be conducted on any scale (i.e. over the whole genome, a set of chromosomes, a single chromosome, a particular subchromosomal region, or any other desired portion of the genome). As mentioned above, once significance thresholds have been assessed, population sample sizes may be adapted as exemplified in Figure 3.

Example 7 below illustrates the increase in statistical power brought to an association study by a haplotype analysis.

The results described in Examples 5 and 7, generated from individual and haplotype studies using a biallelic marker set of an average density equal to ca. 40kb in the region of an Alzheimer's disease trait causing gene, indicate that all biallelic markers of sufficient informative content located within a ca. 200 kb genomic region around a trait-causing allele can potentially be successfully used to localize a trait causing gene with the methods provided by the present invention. This conclusion is further supported by the results obtained through measuring the linkage disequilibrium between markers 99-365-344 or 99-359-308 and ApoE 4 Site A marker within Alzheimer's patients: as one could predict since linkage disequilibrium is the supporting basis for association studies, linkage disequilibrium between these pairs of markers was enhanced in the diseased population vs. the control population. In a similar way as the haplotype analysis enhanced the significance of the corresponding association studies.

Once a given polymorphic site has been found and characterized as a biallelic marker according to the methods of the present invention, several methods can be used in order to determine the specific allele carried by an individual at the given polymorphic base as described in III.

Location of a Gene Associated with Detectable Traits

Once the candidate region has been delineated using the high density biallelic marker map, a sequence analysis process will allow the detection of all genes located within said region, together with a potential functional characterization of said genes. The identified functional features may allow preferred trait-causing candidates to be chosen from among the identified genes. More biallelic markers may then be generated within said candidate genes, and used to perform refined association studies that will support the identification of the trait causing gene. Sequence analysis processes are described in Example 9.

Examples 10-18 illustrate the application of the above methods using biallelic markers to identify a gene associated with a complex disease, prostate cancer, within a ca. 450 kb candidate region. Additional details of the identification of the gene associated with prostate cancer are provided in the U.S. Patent Application entitled "Prostate Cancer Gene" Serial No. 08/996,306.

The above methods were also used to identify biallelic markers in a gene which was an attractive candidate for a gene associated with asthma. Examples 19-26 show how the use of methods of the present invention allowed this gene to be identified as a gene responsible, at least partially, for asthma in the studied populations. Additional details of the identification of the gene associated with asthma are provided in U.S. Provisional Application Serial Nos. 60/081,893.

Alternatively, genes associated with detectable traits may be identified as follows. Candidate genomic regions suspected of harboring a gene associated with the trait may be identified using techniques such as those described herein. In such techniques, the allelic frequencies of biallelic markers are compared in nucleic acid samples derived from individuals expressing the detectable trait and individuals who do not express the detectable trait. In this manner, candidate genomic regions suspected of harboring a gene associated with the detectable trait under investigation are identified.

The existence of one or more genes associated with the detectable trait within the candidate region is confirmed by identifying more biallelic markers lying in the candidate region. A first haplotype analysis is performed for each possible combination of groups of biallelic markers within the genomic region suspected of harboring a trait-associated gene. For example, each group may comprise three biallelic markers. For each of the groups of markers, the frequency of each possible haplotype (for groups of three markers there are 8 possible haplotypes) in individuals expressing the trait and individuals who do not express the trait is estimated. For example, the a haplotype estimation method is applied as described in IV. for example the haplotype frequencies may be estimated using the Expectation-Maximization method of Excoffier L and Slatkin M, *Mol. Biol. Evol.* 12:921-927 (1995).

The frequencies of each of the possible haplotypes of the grouped markers (or each allele of individual markers) in individuals expressing the trait and individuals who do not express the trait are compared. For example, the frequencies may be compared by performing a chi-squared analysis. Within each group, the haplotype (or the allele of each individual marker) having the greatest association with the trait is selected. This process is repeated for each group of biallelic markers (or each allele of the individual markers) to generate a distribution of association values, which will be referred to herein as the "trait-associated" distribution.

A second haplotype analysis is performed for each possible combination of groups of biallelic markers within the genomic regions which are not suspected of harboring a trait-associated gene. For example, each group may comprise three biallelic markers. For each of the groups of markers, the frequency of each possible haplotype (for groups of three markers there are 8 possible haplotypes) in individuals expressing the trait and individuals who do not express the trait is estimated.

The frequencies of each of the possible haplotypes of the grouped markers (or each allele of individual markers) in individuals expressing the trait and individuals who do not express the trait are compared. For example, the frequencies may be compared by performing a chi-squared analysis. Within each group, the haplotype (or the allele of each individual marker) having the greatest association with the trait is selected. This process is repeated for each group of biallelic markers (or each allele of the individual markers) to generate a distribution of association values, which will be referred to herein as the "random" distribution.

The trait-associated distribution and the random distribution are then compared to one another to determine if there are significant differences between them. For example, the trait-associated distribution and the random distribution can be compared using either the Wilcoxon rank test (Noether, G.E. (1991) Introduction to statistics: "The nonparametric way", Springer-Verlag, New York, Berlin) or the Kolmogorov-Smirnov test (Saporta, G. (1990) "Probalites, analyse des donnees et statistiques" Technip editions, Paris) or both the Wilcoxon rank test and the Kolmogorov-Smirnov test.

If the trait-associated distribution and the random distribution are found to be significantly different, the candidate genomic region is highly likely to contain a gene associated with the detectable trait. Accordingly, the candidate genomic region is evaluated more fully to isolate the trait-associated gene. Alternatively, if the trait-associated distribution and the random distribution are equal using the above analyses, the candidate genomic region is unlikely to contain a gene associated with the detectable trait. Accordingly, no further analysis of the candidate genomic region is performed.

While Examples 10 to 26 illustrate the use of the maps and markers of the present invention for identifying a new gene associated with a complex disease within a 2Mb genomic region for establishing that a candidate gene is, at least partially, responsible for a disease, the maps and markers of the present invention may also be used to identify one or more biallelic markers or one or more genes associated with other detectable phenotypes, including drug response, drug toxicity, or drug efficacy. The biallelic markers used in such drug response analyses or shown, using the methods of the present invention to be associated with such traits, may lie within or near genes responsible for or partly responsible for a particular disease, for example a disease against which the drug is meant to act, or may lie within genomic regions which are not responsible for or partly responsible for a disease. In the context of the present invention, a "positive response" to a medicament can be defined as comprising a reduction of the symptoms related to the disease or condition to be treated. In the context of the present invention, a "negative response" to a medicament can be defined as comprising either a lack of positive response to the medicament which does not lead to a symptom reduction or to a side-effect observed following administration of the medicament.

Drug efficacy, response and tolerance/toxicity can be considered as multifactorial traits involving a genetic component in the same way as complex diseases such as Alzheimer's disease, prostate cancer, hypertension or diabetes. As such, the identification of genes involved in drug efficacy and toxicity could be achieved following a positional cloning approach, e.g. performing linkage analysis within families in order to obtain the subchromosomal location of the gene(s). However, this type of analysis is actually impractical in the case of drug responsiveness, due to the lack of availability of familial cases. In fact, the likelihood of having more than one individual in a particular family being exposed to the same drug at the same time is very low. Therefore, drug efficacy and toxicity can only be analyzed as sporadic traits.

In order to conduct association studies to analyze the individual response to a given drug in groups of patients affected with a disease, up to four groups are screened to determine their patterns of biallelic markers using the techniques described above. The four groups are:

- Non-diseased or random controls,
- Diseased patients/drug responders,
- Diseased patients/drug non-responders, and
- Diseased patients/drug side effects.

In preferred embodiments, the above mentioned groups are recruited according to phenotyping criteria having the characteristics described above, so that the phenotypes defining the different groups are non-overlapping, preferably extreme phenotypes. In highly

preferred embodiments, such phenotyping criteria have the bimodal distribution described above.

The final number and composition of the groups for each drug association study is adapted to the distribution of the above described phenotypes within the studied population.

After selecting a suitable population, association and haplotype analyses may be performed as described herein to identify one or more biallelic markers associated with drug response, preferably drug toxicity or drug efficacy. The identification of such one or more biallelic markers allows one to conduct diagnostic tests to determine whether the administration of a drug to an individual will result in drug response, preferably drug toxicity, or drug efficacy.

The methods described above for identifying a gene associated with prostate cancer and biallelic markers indicative of a risk of suffering from asthma may be utilized to identify genes associated with other detectable phenotypes. In particular, the above methods may be used with any marker or combination of markers included in the maps of the present invention, including the biallelic markers of SEQ ID Nos.: 1 to 3809 or the sequences complementary thereto. As described above, the general strategy to perform the association studies using the maps and markers of the present invention is to scan two groups of individuals (trait-positive individuals and trait-negative controls) characterized by a well defined phenotype in order to measure the allele frequencies of the biallelic markers in each of these groups. Preferably, the frequencies of markers with inter-marker spacing of about 150 kb are determined in each group. More preferably, the frequencies of markers with inter-marker spacing of about 75 kb are determined in each group. Even more preferably, markers with inter-marker spacing of about 50 kb, about 37.5kb, about 30kb, or about 25kb will be tested in each population.

In some embodiments the frequenices of 1, 5, 10, 20, 50, 100, 500, 1000, 2000, 3000, or all of the biallelic markers of SEQ ID Nos.: 1 to 3908 or the sequences complementary thereto are measured in each population. In another embodiment, the frequencies of 1, 5, 10, 20, 50, 100, 500, 1000, 2000, or 3000 biallelic markers selected from the group consisting of biallelic markers which are in linkage disequilibrium with the biallelic markers of 1 to 3908 or the sequences complementary thereto are measured in each population. In some embodiments the frequenices of 1, 5, 10, 20, 50, 100, 500, 1000, 2000, or all of the biallelic markers of SEQ ID Nos.: 1 to 2260 or the sequences complementary thereto are measured in each population. In another embodiment, the frequencies of 1, 5, 10, 20, 50, 100, 500, 1000, or 2000 biallelic markers selected from the group consisting of biallelic markers which are in linkage disequilibrium with the biallelic markers of 1 to 2260 or the sequences complementary thereto are measured in each population. In some embodiments the frequenices of 1, 5, 10, 20, 50,

100, 500, 1000, or all of the biallelic markers of SEQ ID Nos.: 2261 to 3734 or the sequences complementary thereto are measured in each population. In another embodiment, the frequencies of 1, 5, 10, 20, 50, 100, 500, 1000 biallelic markers selected from the group consisting of biallelic markers which are in linkage disequilibrium with the biallelic markers of 2261 to 3734 or the sequences complementary thereto are measured in each population. In some embodiments the frequenices of 1, 5, 10, 20, 50, 100, or all of the biallelic markers of SEQ ID Nos.: 3735 to 3908 or the sequences complementary thereto are measured in each population. In another embodiment, the frequencies of 1, 5, 10, 20, 50, or 100 biallelic markers selected from the group consisting of biallelic markers which are in linkage disequilibrium with the biallelic markers of 3735 to 3908 or the sequences complementary thereto are measured in each population.

In some embodiments, the frequencies of about 20,000, or about 40,000 biallelic markers are determined in each population. In a highly preferred embodiment, the frequencies of about 60,000, about 80,000, about 100,000, or about 120,000 biallelic markers are determined in each population. In some embodiments, haplotype analyses may be run using groups of markers located within regions spanning less than 1kb, from 1 to 5kb, from 5 to 10kb, from 10 to 25kb, from 25 to 50kb, from 50 to 150kb, from 150 to 250kb, from 250 to 500kb, from 500kb to 1Mb, or more than 1Mb.

Allele frequency can be measured using any genotyping method described herein including microsequencing techniques; preferred high throughput microsequencing procedures are further exemplified in III; it will be further appreciated that any other large scale genotyping method suitable with the intended purpose contemplated herein may also be used.

It will be appreciated that it is not necessary to use a full high density biallelic marker map in order to start a genome-wide association study. Maps having higher densities of biallelic markers (two or more markers per BAC, average inter-marker spacing of about 75kb or less) may then be generated by starting first on those BACs for which a candidate association has been established at the first step.

In cases when one or more candidate regions have previously been delineated, such as cases where a particular gene or genomic region is suspected of being associated with a trait, local excerpts of biallelic marker maps having densities above one marker per 150kb may be exploited using BACs harboring said genomic regions, or genes, or portions thereof. In these cases also, successive association studies may be performed using sets of biallelic markers showing increasing densities, preferably from about one every 150 kb to about one every 75kb; more preferably, sets of markers with inter-marker spacing below about 50kb, below about 37.5kb, below about 30kb, most preferably below about 25 kb, will be used.

Haplotype analyses may also be conducted using groups of biallelic markers within the candidate region. The biallelic markers included in each of these groups may be located within a genomic region spanning less than 1kb, from 1 to 5kb, from 5 to 10kb, from 10 to 25kb, from 25 to 50kb, from 50 to 150kb, from 150 to 250kb, from 250 to 500kb, from 500kb to 1Mb, or more than 1Mb. It will be appreciated that the ordered DNA fragments containing these groups of biallelic markers need not completely cover the genomic regions of these lengths but may instead be incomplete contigs having one or more gaps therein. As discussed in further detail below, biallelic markers may be used in association studies and haplotype analyses regardless of the completeness of the corresponding physical contig harboring them, provided linkage disequilibrium between the markers can be assessed.

As described above, if a positive association with a trait, such as a disease, or a drug efficacy and/or toxicity, is identified using the biallelic markers and maps of the present invention, the maps will provide not only the confirmation of the association, but also a shortcut towards the identification of the gene involved in the trait under study. As described above, since the markers showing positive association to the trait are in linkage disequilibrium with the trait loci, the causal gene will be physically located in the vicinity of these markers. Regions identified through association studies using high density maps will on average have a 20 - 40 times shorter length than those identified by linkage analysis (2 to 20 Mb).

As described above, once a positive association is confirmed with the high density biallelic marker maps of the present invention, BACs from which the most highly associated markers were derived are completely sequenced and the mutations in the causal gene are searched by applying genomic analysis tools. As described above, once a region harboring a gene associated with a detectable trait has been sequenced and analyzed, the candidate functional regions (e.g. exons and splice sites, promoters and other regulatory regions) are scanned for mutations by comparing the sequences of a selected number of controls and cases, using adequate software.

In some embodiments, trait-positive samples being compared to identify causal mutations are selected among those carrying the ancestral haplotype; in these embodiments, control samples are chosen from individuals not carrying said ancestral haplotype.

In further embodiments, trait-positive samples being compared to identify causal mutations are selected among those showing haplotypes that are as close as possible to the ancestral haplotype; in these embodiments, control samples are chosen from individuals not carrying any of the haplotypes selected for the case population.

The maps and biallelic markers of the present invention may also be used to identify patterns of biallelic markers associated with detectable traits resulting from polygenic interactions. The analysis of genetic interaction between alleles at unlinked loci requires

individual genotyping using the techniques described herein. The analysis of allelic interaction among a selected set of biallelic markers with appropriate p-values can be considered as a haplotype analysis, similar to those described in further details within the present invention.

IX. Use of Biallelic Markers to Identify Individuals Likely to Exhibit a Detectable Trait Associated with a Particular Allele of a Known Gene

In addition to their utility in searches for genes associated with detectable traits on a genome-wide, chromosome-wide, or subchromosomal level, the maps and biallelic markers of the present invention may be used in more targeted approaches for identifying individuals likely to exhibit a particular detectable trait or individuals who exhibit a particular detectable trait as a consequence of possessing a particular allele of a gene associated with the detectable trait. For example, the biallelic markers and maps of the present invention may be used to identify individuals who carry an allele of a known gene that is suspected of being associated with a particular detectable trait. In particular, the target genes may be genes having alleles which predispose an individual to suffer from a specific disease state. In other cases, the target genes may be genes having alleles that predispose an individual to exhibit a desired or undesired response to a drug or other pharmaceutical composition, a food, or any administered compound. The known gene may encode any of a variety of types of biomolecules. For example, the known genes targeted in such analyzes may be genes known to be involved in a particular step in a metabolic pathway in which disruptions may cause a detectable trait. Alternatively, the target genes may be genes encoding receptors or ligands which bind to receptors in which disruptions may cause a detectable trait, genes encoding transporters, genes encoding proteins with signaling activities, genes encoding proteins involved in the immune response, genes encoding proteins involved in hematopoesis, or genes encoding proteins involved in wound healing. It will be appreciated that the target genes are not limited to those specifically enumerated above, but may be any gene known to be or suspected of being associated with a detectable trait.

As previously mentioned, the maps and markers of the present invention may be used to identify genes associated with drug response. The biallelic markers of the present invention may also be used to select individuals for inclusion in the clinical trials of a drug. In some embodiments, the markers of SEQ ID Nos.: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto may be used in targeted approaches to identify individuals at risk of developing a detectable trait, for example a complex disease or desired/undesired drug response, or to identify individuals exhibiting said trait. The present invention provides methods to establish putative associations between any of the biallelic markers described herein and any detectable traits, including those specifically described herein.

To use the maps and markers of the present invention in further targeted approaches, biallelic markers which are in linkage disequilibrium with any of the above disclosed markers may be identified. In cases where one or more biallelic markers of the present invention have been shown to be associated with a detectable trait, more biallelic markers in linkage disequilibrium with said associated biallelic markers may be generated and used to perform targeted approaches aiming at identifying individuals exhibiting, or likely to exhibit, said detectable trait, according to the methods provided herein.

Furthermore, in cases where a candidate gene is suspected of being associated with a particular detectable trait or suspected of causing the detectable trait, biallelic markers in linkage disequilibrium with said candidate gene may be identified and used in targeted approaches, such as the approaches utilized above for the asthma-associated gene and the Apo E gene.

Biallelic markers that are in linkage disequilibrium with markers associated with a detectable trait, or with genes associated with a detectable trait, or suspected of being so, are identified by performing single marker analyzes, haplotype association analyzes, or linkage disequilibrium measurements on samples from trait-positive and trait-negative individuals as described above using biallelic markers lying in the vicinity of the target marker or gene. In this manner, a single biallelic marker or a group of biallelic markers may be identified which indicate that an individual is likely to possess the detectable trait or does possess the detectable trait as a consequence of a particular allele of the target marker or gene.

Nucleic acid samples from individuals to be tested for predisposition to a detectable trait or possession of a detectable trait as a consequence of a particular allele of the target gene may be examined using the diagnostic methods described above.

Throughout this application, various publications, patents, and published patent applications are cited. The disclosures of the publications, patents, and published patent specifications referenced in this application are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

EXAMPLES

Several of the methods of the present invention are described in the following examples, which are offered by way of illustration and not by way of limitation. Many other modifications and variations of the invention as herein set forth can be made without departing from the spirit and scope thereof and therefore only such limitations should be imposed as are indicated by the appended claims.

Example 1

Ordering of a BAC Library: Screening Clones with STSs

The BAC library is screened with a set of PCR-typeable STSs to identify clones containing the STSs. To facilitate PCR screening of several thousand clones, for example 200,000 clones, pools of clones are prepared.

Three-dimensional pools of the BAC libraries are prepared as described in Chumakov et al. and are screened for the ability to generate an amplification fragment in amplification reactions conducted using primers derived from the ordered STSs. (Chumakov et al. (1995), *supra*). A BAC library typically contains 200,000 BAC clones. Since the average size of each insert is 100-300 kb, the overall size of such a library is equivalent to the size of at least about 7 human genomes. This library is stored as an array of individual clones in 518 384-well plates. It can be divided into 74 primary pools (7 plates each). Each primary pool can then be divided into 48 subpools prepared by using a three-dimensional pooling system based on the plate, row and column address of each clone (more particularly, 7 subpools consisting of all clones residing in a given microtiter plate; 16 subpools consisting of all clones in a given row; 24 subpools consisting of all clones in a given column).

Amplification reactions are conducted on the pooled BAC clones using primers specific for the STSs. For example, the three dimensional pools may be screened with 45,000 STSs whose positions relative to one another and locations along the genome are known. Preferably, the three dimensional pools are screened with about 30,000 STSs whose positions relative to one another and locations along the genome are known. In a highly preferred embodiment, the three dimensional pools are screened with about 20,000 STSs whose positions relative to one another and locations along the genome are known.

Amplification products resulting from the amplification reactions are detected by conventional agarose gel electrophoresis combined with automatic image capturing and processing. PCR screening for a STS involves three steps: (1) identifying the positive primary pools; (2) for each positive primary pool, identifying the positive plate, row and column 'subpools' to obtain the address of the positive clone; (3) directly confirming the PCR assay on the identified clone. PCR assays are performed with primers specifically defining the STS.

Screening is conducted as follows. First BAC DNA containing the genomic inserts is prepared as follows. Bacteria containing the BACs are grown overnight at 37°C in 120 µl of LB containing chloramphenicol (12 µg/ml). DNA is extracted by the following protocol:

Centrifuge 10 min at 4°C and 2000 rpm

Eliminate supernatant and resuspend pellet in 120 µl TE 10-2 (Tris HCl 10 mM, EDTA 2 mM)

Centrifuge 10 min at 4°C and 2000 rpm

Eliminate supernatant and incubate pellet with 20 µl lyzozyme 1 mg/ml during 15 min at room temperature

Add 20 µl proteinase K 100µg/ml and incubate 15 min at 60°C

Add 8 µl DNAse 2U/µl and incubate 1 hr at room temperature

Add 100 µl TE 10-2 and keep at –80°C

PCR assays are performed using the following protocol:

Final volume	15 µl
BAC DNA	1.7 ng/µl
$MgCl_2$	2 mM
dNTP (each)	200 µM
primer (each)	2.9 ng/µl
Ampli Taq Gold DNA polymerase	0.05 unit/µl
PCR buffer (10x = 0.1 M TrisHCl pH8.3 0.5M KCl	1x

The amplification is performed on a Genius II thermocycler. After heating at 95°C for 10 min, 40 cycles are performed. Each cycle comprises: 30 sec at 95°C, 54°C for 1 min, and 30 sec at 72°C. For final elongation, 10 min at 72°C end the amplification. PCR products are analyzed on 1% agarose gel with 0.1 mg/ml ethidium bromide.

Alternatively, a YAC (Yeast Artificial Chromosome) library can be used. The very large insert size, of the order of 1 megabase, is the main advantage of the YAC libraries. The library can typically include about 33,000 YAC clones as described in Chumakov et al. (1995, *supra*). The YAC screening protocol may be the same as the one used for BAC screening.

The known order of the STSs is then used to align the BAC inserts in an ordered array (contig) spanning the whole human genome. If necessary new STSs to be tested can be generated by sequencing the ends of selected BAC inserts. Subchromosomal localization of the BACs can be established and/or verified by fluorescence in situ hybridization (FISH), performed on metaphasic chromosomes as described by Cherif et al. 1990 and in Example 3 below. BAC insert size may be determined by Pulsed Field Gel Electrophoresis after digestion with the restriction enzyme NotI.

Finally, a minimally overlapping set of BAC clones, with known insert size and subchromosomal location, covering the entire genome, a set of chromosomes, a single chromosome, a particular subchromosomal region, or any other desired portion of the genome is selected from the DNA library. For example, the BAC clones may cover at least 100kb of contiguous genomic DNA, at least 250kb of contiguous genomic DNA, at least 500kb of contiguous genomic DNA, at least 2Mb of contiguous genomic DNA, at least 5Mb of

contiguous genomic DNA, at least 10Mb of contiguous genomic DNA, or at least 20Mb of contiguous genomic DNA.

Example 2

Screening BAC libraries with biallelic markers

Amplification primers enabling the specific amplification of DNA fragments carrying the biallelic markers, including the map-related biallelic markers of the invention, may be used to screen clones in any genomic DNA library, preferably the BAC libraries described above for the presence of the biallelic markers.

Pairs of primers of SEQ ID Nos: 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 were designed which allow the amplification of fragments carrying the biallelic markers of SEQ ID Nos: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto. The amplification primers of SEQ ID Nos: 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773 may be used to screen clones in a genomic DNA library for the presence of the biallelic markers of SEQ ID Nos: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto.

It will be appreciated that amplification primers for the biallelic markers of SEQ ID Nos: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 need not be identical to the primers of SEQ ID Nos: 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773. Rather, they can be any other primers allowing the specific amplification of any DNA fragment carrying the markers and may be designed using techniques familiar to those skilled in the art. The amplification primers may be oligonucleotides of 8, 10, 15, 20 or more bases in length which enable the amplification of any fragment carrying the polymorphic site in the markers. The polymorphic base may be in the center of the amplification product or, alternatively, it may be located off-center. For example, in some embodiments, the amplification product produced using these primers may be at least 100 bases in length (i.e. 50 nucleotides on each side of the polymorphic base in amplification products in which the polymorphic base is centrally located). In other embodiments, the amplification product produced using these primers may be at least 500 bases in length (i.e. 250 nucleotides on each side of the polymorphic base in amplification products in which the polymorphic base is centrally located). In still further embodiments, the amplification product produced using these primers may be at least 1000 bases in length (i.e. 500 nucleotides on each side of the polymorphic base in amplification products in which the polymorphic base is centrally located). Amplification primers such as those described above are included within the scope of the present invention.

The localization of biallelic markers on BAC clones is performed essentially as

described in Example 1.

The BAC clones to be screened are distributed in three dimensional pools as described in Example 1.

Amplification reactions are conducted on the pooled BAC clones using primers specific for the biallelic markers to identify BAC clones which contain the biallelic markers, using procedures essentially similar to those described in Example 1.

Amplification products resulting from the amplification reactions are detected by conventional agarose gel electrophoresis combined with automatic image capturing and processing. PCR screening for a biallelic marker involves three steps: (1) identifying the positive primary pools; (2) for each positive primary pools, identifying the positive plate, row and column 'subpools' to obtain the address of the positive clone; (3) directly confirming the PCR assay on the identified clone. PCR assays are performed with primers defining the biallelic marker.

Screening is conducted as follows. First BAC DNA is isolated as follows. Bacteria containing the genomic inserts are grown overnight at 37°C in 120 µl of LB containing chloramphenicol (12 µg/ml). DNA is extracted by the following protocol:

Centrifuge 10 min at 4°C and 2000 rpm

Eliminate supernatant and resuspend pellet in 120 µl TE 10-2 (Tris HCl 10 mM, EDTA 2 mM)

Centrifuge 10 min at 4°C and 2000 rpm

Eliminate supernatant and incubate pellet with 20 µl lyzozyme 1 mg/ml during 15 min at room temperature

Add 20 µl proteinase K 100µg/ml and incubate 15 min at 60°C

Add 8 µl DNAse 2U/µl and incubate 1 hr at room temperature

Add 100 µl TE 10-2 and keep at –80°C

PCR assays are performed using the following protocol:

Final volume	15 µl
BAC DNA	1.7 ng/µl
$MgCl_2$	2 mM
dNTP (each)	200 µM
primer (each)	2.9 ng/µl
Ampli Taq Gold DNA polymerase	0.05 unit/µl
PCR buffer (10x = 0.1 M TrisHCl pH8.3 0.5M KCl	1x

The amplification is performed on a Genius II thermocycler. After heating at 95°C for 10 min, 40 cycles are performed. Each cycle comprises: 30 sec at 95°C, 54°C for 1 min, and 30 sec at 72°C. For final elongation, 10 min at 72°C end the amplification. PCR products are analyzed on 1% agarose gel with 0.1 mg/ml ethidium bromide.

Example 3

Assignment of Biallelic Markers to Subchromosomal Regions

Metaphase chromosomes are prepared from phytohemagglutinin (PHA)-stimulated blood cell donors. PHA-stimulated lymphocytes from healthy males are cultured for 72 h in RPMI-1640 medium. For synchronization, methotrexate (10 mM) is added for 17 h, followed by addition of 5-bromodeoxyuridine (5-BudR, 0.1 mM) for 6 h. Colcemid (1 mg/ml) is added for the last 15 min before harvesting the cells. Cells are collected, washed in RPMI, incubated with a hypotonic solution of KCl (75 mM) at 37°C for 15 min and fixed in three changes of methanol:acetic acid (3:1). The cell suspension is dropped onto a glass slide and air-dried.

BAC clones carrying the biallelic markers used to construct the maps of the present invention (including the biallelic markers of SEQ ID Nos: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto) can be isolated as described above. These BACs or portions thereof, including fragments carrying said biallelic markers, obtained for example from amplification reactions using pairs of primers of SEQ ID Nos: 3935 to 7842, 3935 to 6194, 6195 to 7668, 7669 to 7842, 7866 to 11773, 7866 to 10125, 10126 to 11599, and 11600 to 11773, can be used as probes to be hybridized with metaphasic chromosomes. It will be appreciated that the hybridization probes to be used in the contemplated method may be generated using alternative methods well known to those skilled in the art. Hybridization probes may have any length suitable for this intended purpose.

Probes are then labeled with biotin-16 dUTP by nick translation according to the manufacturer's instructions (Bethesda Research Laboratories, Bethesda, MD), purified using a Sephadex G-50 column (Pharmacia, Upssala, Sweden) and precipitated. Just prior to hybridization, the DNA pellet is dissolved in hybridization buffer (50% formamide, 2 X SSC, 10% dextran sulfate, 1 mg/ml sonicated salmon sperm DNA, pH 7) and the probe is denatured at 70°C for 5-10 min.

Slides kept at -20°C are treated for 1 h at 37°C with RNase A (100 mg/ml), rinsed three times in 2 X SSC and dehydrated in an ethanol series. Chromosome preparations are denatured in 70% formamide, 2 X SSC for 2 min at 70°C, then dehydrated at 4°C. The slides are treated with proteinase K (10 mg/100 ml in 20 mM Tris-HCl, 2 mM $CaCl_2$) at 37°C for 8 min and dehydrated. The hybridization mixture containing the probe is placed on the slide, covered with a coverslip, sealed with rubber cement and incubated overnight in a humid chamber at 37°C. After hybridization and post-hybridization washes, the biotinylated probe is detected by avidin-

FITC and amplified with additional layers of biotinylated goat anti-avidin and avidin-FITC. For chromosomal localization, fluorescent R-bands are obtained as previously described (Cherif et al.,(1990) *supra.*). The slides are observed under a LEICA fluorescence microscope (DMRXA). Chromosomes are counterstained with propidium iodide and the fluorescent signal of the probe appears as two symmetrical yellow-green spots on both chromatids of the fluorescent R-band chromosome (red). Thus, a particular biallelic marker may be localized to a particular cytogenetic R-band on a given chromosome.

The above procedure was used to confirm the subchromosomal location of many of the BAC clones harboring the markers obtained above. In particular, several of the markers were assigned to subchromosomal regions of chromosome 21. Simple identification numbers were attributed to each BAC from which the markers are derived. Figure 1 is a cytogenetic map of chromosome 21 indicating the subchromosomal regions therein. Amplification primers for generating amplification products containing the polymorphic bases of these markers are also provided in the accompanying sequence listing. In addition, microsequencing primers for use in determining the identities of the polymorphic bases of these biallelic markers are provided in the accompanying Sequence Listing.

The rate at which biallelic markers may be assigned to subchromosomal regions may be enhanced through automation. For example, probe preparation may be performed in a microtiter plate format, using adequate robots. The rate at which biallelic markers may be assigned to subchromosomal regions may be enhanced using techniques which permit the *in situ* hybridization of multiple probes on a single microscope slide, such as those disclosed in Larin et al., *Nucleic Acids Research* 22: 3689-3692 (1994). In the largest test format described, different probes were hybridized simultaneously by applying them directly from a 96-well microtiter dish which was inverted on a glass plate. Software for image data acquisition and analysis that is adapted to each optical system, test format, and fluorescent probe used, can be derived from the system described in Lichter et al. *Science* 247: 64-69 (1990). Such software measures the relative distance between the center of the fluorescent spot corresponding to the hybridized probe and the telomeric end of the short arm of the corresponding chromosome, as compared to the total length of the chromosome. The rate at which biallelic markers are assigned to subchromosomal locations may be further enhanced by simultaneously applying probes labeled with different flouorescent tags to each well of the 96 well dish. A further benefit of conducting the analysis on one slide is that it facilitates automation, since a microscope having a moving stage and the capability of detecting fluorescent signals in different metaphase chromosomes could provide the coordinates of each probe on the metaphase chromosomes distributed on the 96 well dish.

Example 4 below describes an alternative method to position biallelic markers which

allows their assignment to human chromosomes.

Example 4

Assignment of Biallelic Markers to Human Chromosomes

The biallelic markers used to construct the maps of the present invention, including the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto, may be assigned to a human chromosome using monosomal analysis as described below.

The chromosomal localization of a biallelic marker can be performed through the use of somatic cell hybrid panels. For example 24 panels, each panel containing a different human chromosome, may be used (Russell et al., *Somat Cell Mol.* Genet 22:425-431 (1996); Drwinga et al., Genomics 16:311-314 (1993)).

The biallelic markers are localized as follows. The DNA of each somatic cell hybrid is extracted and purified. Genomic DNA samples from a somatic cell hybrid panel are prepared as follows. Cells are lysed overnight at 42°C with 3.7 ml of lysis solution composed of:

3 ml TE 10-2 (Tris HCl 10 mM, EDTA 2 mM) / NaCl 0.4 M

200 µl SDS 10%

500 µl K-proteinase (2 mg K-proteinase in TE 10-2 / NaCl 0.4 M)

For the extraction of proteins, 1 ml saturated NaCl (6M) (1/3.5 v/v) is added. After vigorous agitation, the solution is centrifuged for 20 min at 10,000 rpm. For the precipitation of DNA, 2 to 3 volumes of 100 % ethanol are added to the previous supernatant, and the solution is centrifuged for 30 min at 2,000 rpm. The DNA solution is rinsed three times with 70 % ethanol to eliminate salts, and centrifuged for 20 min at 2,000 rpm. The pellet is dried at 37°C, and resuspended in 1 ml TE 10-1 or 1 ml water. The DNA concentration is evaluated by measuring the OD at 260 nm (1 unit OD = 50 µg/ml DNA). To determine the presence of proteins in the DNA solution, the OD_{260}/OD_{280} ratio is determined. Only DNA preparations having a OD_{260}/OD_{280} ratio between 1.8 and 2 are used in the PCR assay.

Then, a PCR assay is performed on genomic DNA with primers defining the biallelic marker. The PCR assay is performed as described above for BAC screening. The PCR products are analyzed on a 1% agarose gel containing 0.2 mg/ml ethidium bromide.

Example 5

Measurement of Linkage Disequilibrium

As originally reported by Strittmatter et al. and by Saunders et al. in 1993, the Apo E e4 allele is strongly associated with both late-onset familial and sporadic Alzheimer's disease. (Saunders, A.M. Lancet 342: 710-711 (1993) and Strittmater, W.J. et al., Proc. Natl. Acad. Sci. U.S.A. 90: 1977-1981 (1993)). The 3 major isoforms of human Apolipoprotein E (apoE2,

-E3, and -E4), as identified by isoelectric focusing, are coded for by 3 alleles (e 2, 3, and 4). The e 2, e 3, and e 4 isoforms differ in amino acid sequence at 2 sites, residue 112 (called site A) and residue 158 (called site B). The ancestral isoform of the protein is Apo E3, which at sites A/B contains cysteine/arginine, while ApoE2 and -E4 contain cysteine/cysteine and arginine/arginine, respectively (Weisgraber, K.H. et al., J. Biol. Chem. 256: 9077-9083 (1981); Rall, S.C. et al., Proc. Natl. Acad. Sci. U.S.A. 79: 4696-4700 (1982)).

Apo E e 4 is currently considered as a major susceptibility risk factor for Alzheimer's disease development in individuals of different ethnic groups (specially in Caucasians and Japanese compared to Hispanics or African Americans), across all ages between 40 and 90 years, and in both men and women, as reported recently in a study performed on 5930 Alzheimer's disease patients and 8607 controls (Farrer et al., *JAMA* 278:1349-1356 (1997)). More specifically, the frequency of a C base coding for arginine 112 at site A is significantly increased in Alzheimer's disease patients.

Although the mechanistic link between Apo E e 4 and neuronal degeneration characteristic of Alzheimer's disease remains to be established, current hypotheses suggest that the Apo E genotype may influence neuronal vulnerability by increasing the deposition and/or aggregation of the amyloid beta peptide in the brain or by indirectly reducing energy availability to neurons by promoting atherosclerosis.

Using the methods of the present invention, biallelic markers that are in the vicinity of the Apo E site A were generated and the association of one of their alleles with Alzheimer's disease was analyzed. An Apo E public marker (stSG94) was used to screen a human genome BAC library as previously described. A BAC, which gave a unique FISH hybridization signal on chromosomal region 19q13.2.3, the chromosomal region harboring the Apo E gene, was selected for finding biallelic markers in linkage disequilibrium with the Apo E gene as follows.

This BAC contained an insert of 205 kb that was subcloned as previously described. Fifty BAC subclones were randomly selected and sequenced. Twenty five subclone sequences were selected and used to design twenty five pairs of PCR primers allowing 500 bp-amplicons to be generated. These PCR primers were then used to amplify the corresponding genomic sequences in a pool of DNA from 100 unrelated individuals (blood donors of French origin) as already described.

Amplification products from pooled DNA were sequenced and analyzed for the presence of biallelic polymorphisms, as already described. Five amplicons were shown to contain a polymorphic base in the pool of 100 unrelated individuals, and therefore these polymorphisms were selected as random biallelic markers in the vicinity of the Apo E gene. The sequences of both alleles of these biallelic markers (99-344-439; 99-366-274, 99-359-308;

99-355-219; 99-365-344;) correspond to SEQ ID Nos: 3909 to 3913. Corresponding pairs of amplification primers for generating amplicons containing these biallelic markers can be chosen from those listed as SEQ ID Nos: 7843 to 7847 and 11774 to 11778.

An additional pair of primers (SEQ ID Nos: 3124 and 4169) was designed that allows amplification of the genomic fragment carrying the biallelic polymorphism corresponding to the ApoE marker (99-2452-54; C/T; designated SEQ ID NO: 3914 in the accompanying Sequence Listing; publicly known as Apo E site A (Weisgraber et al. (1981), *supra*; Rall et al. (1982), *supra*) to be amplified.

The five random biallelic markers plus the Apo E site A marker were physically ordered by PCR screening of the corresponding amplicons using all available BACs originally selected from the genomic DNA libraries, as previously described, using the public Apo E marker stSG94. The amplicon's order derived from this BAC screening is as follows: (99-344-439/99-366-274) - (99-365-344/99-2452-54) - 99-359-308 - 99-355-219, where parentheses indicate that the exact order of the respective amplicons couldn't be established.

Linkage disequilibrium among the six biallelic markers (five random markers plus the Apo E site A) was determined by genotyping the same 100 unrelated individuals from whom the random biallelic markers were identified.

DNA samples and amplification products from genomic PCR were obtained in similar conditions as those described above for the generation of biallelic markers, and subjected to automated microsequencing reactions using fluorescent ddNTPs (specific fluorescence for each ddNTP) and the appropriate microsequencing primers having a 3' end immediately upstream of the polymorphic base in the biallelic markers. Once specifically extended at the 3' end by a DNA polymerase using the complementary fluorescent dideoxynucleotide analog (thermal cycling), the microsequencing primer was precipitated to remove the unincorporated fluorescent ddNTPs. The reaction products were analyzed by electrophoresis on ABI 377 sequencing machines. Results were automatically analyzed by an appropriate software further described in Example 8.

Linkage disequilibrium (LD) between all pairs of biallelic markers (Mi, Mj) was calculated for every allele combination (Mi1,Mj1 ; Mi1,Mj2 ; Mi2,Mj1 ; Mi2,Mj2) according to the maximum likelihood estimate (MLE) for delta (the composite linkage disequilibrium coefficient). The results of the linkage disequilibrium analysis between the Apo E Site A marker and the five new biallelic markers (99-344-439 ; 99-355-219 ; 99-359-308 ; 99-365-344 ; 99-366-274) are summarized in Table 2 below:

Table 2

Markers	d x 100	SEQ ID Nos of the biallelic Markers	SEQ ID Nos of the amplification Primers
ApoE SiteA	1028	3124	
99-2452-54	2076	4169	
99-344-439	1	1023	3119
	2071	4164	
99-366-274	1	1024	3120
	2072	4165	
99-365-344	8	1027	3123
	2075	4168	
99-359-308	2	1025	3121
	2073	4166	
99-355-219	1	1026	3122
	2074	4167	

The above linkage disequilibrium results indicate that among the five biallelic markers randomly selected in a region of about 200 kb containing the Apo E gene, marker 99-365-344T is in relatively strong linkage disequilibrium with the Apo E site A allele (99-2452-54C).

Therefore, since the Apo E site A allele is associated with Alzheimer's disease, one can predict that the T allele of marker 99-365-344 will probably be found associated with Alzheimer's disease. In order to test this hypothesis, the biallelic markers of SEQ ID Nos: 3909 to 3913 were used in association studies as described below.

225 Alzheimer's disease patients were recruited according to clinical inclusion criteria based on the MMSE test. The 248 control cases included in this study were both ethnically- and age-matched to the affected cases. Both affected and control individuals corresponded to unrelated cases. The identities of the polymorphic bases of each of the biallelic markers was determined in each of these individuals using the methods described above. Techniques for conducting association studies are further described below.

The results of this study are summarized in Table 3 below :

Table 3

MARKER	ASSOCIATION DATA	
	Difference in allele frequency between individuals with Alzheimer's and control individuals	Corresponding p-value
99-344-439	3.3 %	9.54 E-02
99-366-274	1.6%	2.09 E-01
99-365-344	**17.7%**	**6.9 E-10**
99-2452-54 (ApoE Site A)	**23.8%**	**3.95 E-21**
99-359-308	0.4%	9.2 E-01
99-355-219	2.5%	2.54 E-01

The frequency of the Apo E site A allele in both Alzheimer's disease cases and controls was found in agreement with that previously reported (ca. 10% in controls and ca. 34% in Alzheimer's disease cases, leading to a 24% difference in allele frequency), thus validating the Apo E e4 association in the populations used for this study.

Moreover, as predicted from the linkage disequilibrium analysis (Table 3), a significant association of the T allele of marker 99-365/344 with Alzheimer's disease cases (18% increase in the T allele frequency in Alzheimer's disease cases compared to controls, p value for this difference = 6.9 E-10) was observed.

The above results indicate that any marker in linkage disequilibrium with one given marker associated with a trait will be associated with the trait. It will be appreciated that, though in this case the ApoE Site A marker is the trait-causing allele (TCA) itself, the same conclusion could be drawn with any other non trait-causing allele marker associated with the studied trait.

These results further indicate that conducting association studies with a set of biallelic markers randomly generated within a candidate region at a sufficient density (here about one biallelic marker every 40kb on average), allows the identification of at least one marker associated with the trait.

In addition, these results correlate with the physical order of the six biallelic markers contemplated within the present example (see above) : marker 99-365/344, which had been found to be the closest in terms of physical distance to the ApoE Site A marker, also shows the strongest linkage disequilibrium with the Apo E site A marker.

In order to further refine the relationship between physical distance and linkage disequilibrium between biallelic markers, a ca. 450 kb fragment from a genomic region on chromosome 8 was fully sequenced.

LD within ca. 230 pairs of biallelic markers derived therefrom was measured in a random French population and analyzed as a function of the known physical inter-marker spacing. This analysis confirmed that, on average, linkage disequilibrium between 2 biallelic markers correlates with the physical distance that separates them. It further indicated that linkage disequilibrium between 2 biallelic markers tends to decrease when their spacing increases. More particularly, linkage disequilibrium between 2 biallelic markers tends to decrease when their inter-marker distance is greater than 50kb, and is further decreased when the inter-marker distance is greater than 75kb. It was further observed that when 2 biallelic markers were further than 150kb apart, most often no significant linkage disequilibrium between them could be evidenced. It will be appreciated that the size and history of the sample population used to measure linkage disequilibrium between markers may influence the distance beyond which linkage disequilibrium tends not to be detectable.

Assuming that linkage disequilibrium can be measured between markers spanning regions up to an average of 150kb long, biallelic marker maps will allow genome-wide linkage disequilibrium mapping, provided they have an average inter-marker distance lower than 150kb.

Example 6

Identification of a Candidate Region Harboring a Gene Associated with a Detectable Trait

The initial identification of a candidate genomic region harboring a gene associated with a detectable trait may be conducted using a genome-wide map comprising about 20,000 biallelic markers. The candidate genomic region may be further defined using a map having a higher marker density, such as a map comprising about 40,000 markers, about 60,000 markers, about 80,000 markers, about 100,000 markers, or about 120,000 markers.

The use of high density maps such as those described above allows the identification of genes which are truly associated with detectable traits, since the coincidental associations will be randomly distributed along the genome while the true associations will map within one or more discrete genomic regions. Accordingly, biallelic markers located in the vicinity of a gene associated with a detectable trait will give rise to broad peaks in graphs plotting the frequencies of the biallelic markers in trait-positive individuals versus control individuals. In contrast, biallelic markers which are not in the vicinity of the gene associated with the detectable trait will produce unique points in such a plot. By determining the association of several markers within the region containing the gene associated with the detectable trait, the gene associated with the detectable trait can be identified using an association curve which reflects the difference between the allele frequencies within the trait-positive and control

populations for each studied marker. The gene associated with the detectable trait will be found in the vicinity of the marker showing the highest association with the trait.

Figures 4, 5, and 6 provide a simulated illustration of the above principles. As illustrated in Figure 4, an association analysis conducted with a map comprising about 3,000 biallelic markers yields a group of points. However, when an association analysis is performed using a denser map which includes additional biallelic markers, the points become broad peaks indicative of the location of a gene associated with a detectable trait. For example, the biallelic markers used in the initial association analysis may be obtained from a map comprising about 20,000 biallelic markers, as illustrated by the simulation results shown in Figure 5. In some embodiments, one or more of the biallelic markers of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto are used in the association analysis.

In the simulated results of Figure 4, the association analysis with 3,000 markers suggests peaks near markers 9 and 17.

Next, a second analysis is performed using additional markers in the vicinity of markers 9 and 17, as illustrated in the simulated results of Figure 5, using a map of about 20,000 markers. This step again indicates an association in the close vicinity of marker 17, since more markers in this region show an association with the trait. However, none of the additional markers around marker 9 shows a significant association with the trait, which makes marker 9 a potential false positive. In some embodiments, one or more of the biallelic markers selected from the group consisting of SEQ ID Nos. 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto are used in the second analysis. In order to further test the validity of these two suspected associations, a third analysis may be obtained with a map comprising about 60,000 biallelic markers. In some embodiments, one or more of the biallelic markers selected from the group consisting of SEQ ID Nos: 1 to 3908, 1 to 2260, 2261 to 3374, 3735 to 3908 or the sequences complementary thereto are used in the third association analysis. In the simulated results of Figure 6, more markers lying around marker 17 exhibit a high degree of association with the detectable trait. Conversely, no association is confirmed in the vicinity of marker 9. The genomic region surrounding marker 17 can thus be considered a candidate region for the potential trait of this simulation.

Example 7

Haplotype Analysis: Identification of biallelic markers delineating a genomic region associated with Alzheimer's Disease (AD)

As shown in Table 3 within Example 5, at an average map density of one marker per 40 kb only one marker (99-365-344) out of five random biallelic markers from a ca. 200 kb genomic region around the Apo E gene showed a clear association to Alzheimer's disease

(delta allelic frequency in cases and controls =18% ; p value = 6.9 E-10). The allelic frequencies of the other four random markers were not significantly different between Alzheimer's disease cases and controls (p-values ≥ E-01). However, since linkage disequilibrium can usually be detected between markers located further apart than an average 40 kb as previously discussed, one should expect that, performing an association study with a local excerpt of a biallelic marker map covering ca. 200kb with an average inter-marker distance of ca. 40kb should allow the identification of more than one biallelic marker associated with Alzheimer's disease.

A haplotype analysis was thus performed using the biallelic markers 99-344-439; 99-355-219; 99-359-308; 99-365-344; and 99-366-274 (of SEQ ID Nos: 3909 to 3919).

In a first step, marker 99-365-344 that was already found associated with Alzheimer's disease was not included in the haplotype study. Only biallelic markers 99-344-439, 99-355-219, 99-359-308, and 99-366-274, which did not show any significant association with Alzheimer's disease when taken individually, were used. This first haplotype analysis measured frequencies of all possible two-, three-, or four-marker haplotypes in the Alzheimer's disease case and control populations. As shown in Figure 7, there was one haplotype among all the potential different haplotypes based on the four individually non-significant markers ("haplotype 8", TAGG comprising SEQ ID No. 3910 with the T allele of marker 99-366-274, SEQ ID No. 3909 with the A allele of marker 99-344-439, SEQ ID No. 3911 with the G allele of marker 99-359-308 and SEQ ID No. 3912 which is the G allele of marker 99-355-219), that was present at statistically significant different frequencies in the Alzheimer's disease case and control populations (D=12% ; p value = 2.05 E-06). Moreover, a significant difference was already observed for a three-marker haplotype included in the above mentioned "haplotype 8" ("haplotype 7", TGG, D=10% ; p value = 4.76 E-05). Haplotype 7 comprises SEQ ID No. 3910 with the T allele of marker 99-366-274, SEQ ID No. 3911 with the G allele of marker 99-359-308 and SEQ ID No. 3912 with the G allele of marker 99-355-219). The haplotype association analysis thus clearly increased the statistical power of the individual marker association studies by more than four orders of magnitude when compared to single-marker analysis from p values ≥ E-01 for the individual markers to p value ≤ 2 E-06 for the four-marker "haplotype 8". See Table 3.

The significance of the values obtained for this haplotype association analysis was evaluated by the following computer simulation. The genotype data from the Alzheimer's disease cases and the unaffected controls were pooled and randomly allocated to two groups which contained the same number of individuals as the case/control groups used to produce the data summarized in Figure 7. A four-marker haplotype analysis (99-344-439 ; 99-355-219 ; 99-359-308 ; and 99-366-274) was run on these artificial groups. This experiment was

reiterated 100 times and the results are shown in Figure 8. No haplotype among those generated was found for which the p-value of the frequency difference between both populations was more significant than 1 E-05. In addition, only 4% of the generated haplotypes showed p-values lower than 1 E-04. Since both these p-value thresholds are less significant than the 2 E-06 p-value showed by "haplotype 8", this haplotype can be considered significantly associated with Alzheimer's disease.

In a second step, marker 99-365-344 was included in the haplotype analyzes. The frequency differences between the affected and non affected populations was calculated for all two-, three-, four- or five-marker haplotypes involving markers: 99-344-439; 99-355-219; 99-359-308; 99-366-274; and 99-365-344. The most significant p-values obtained in each category of haplotype (involving two, three, four or five markers) were examined depending on which markers were involved or not within the haplotype. This showed that all haplotypes which included marker 99-365-344 showed a significant association with Alzheimer's disease (p-values in the range of E-04 to E-11).

An additional way of evaluating the significance of the values obtained in the haplotype association analysis was to perform a similar Alzheimer's disease case-control study on biallelic markers generated from BACs containing inserts corresponding to genomic regions derived from chromosomes 13 or 21 and not known to be involved in Alzheimer's disease. Performing similar haplotype and individual association analyzes as those described above and in Example 10 did not generate any significant association results (all p-values for haplotype analyzes were less significant than E-03; all p-values for single marker association studies were less significant than E-02).

Example 8

Genotyping of biallelic markers using microsequencing procedures

Several microsequencing protocols conducted in liquid phase are well known to those skilled in the art. A first possible detection analysis allowing the allele characterization of the microsequencing reaction products relies on detecting fluorescent ddNTP- extended microsequencing primers after gel electrophoresis. A first alternative to this approach consists in performing a liquid phase microsequencing reaction, the analysis of which may be carried out in solid phase.

For example, the microsequencing reaction may be performed using 5'-biotinylated oligonucleotide primers and fluorescein-dideoxynucleotides. The biotinylated oligonucleotide is annealed to the target nucleic acid sequence immediately adjacent to the polymorphic nucleotide position of interest. It is then specifically extended at its 3'-end following a PCR cycle, wherein the labeled dideoxynucleotide analog complementary to the polymorphic base is incorporated. The biotinylated primer is then captured on a microtiter plate coated with

streptavidin. The analysis is thus entirely carried out in a microtiter plate format. The incorporated ddNTP is detected by a fluorescein antibody - alkaline phosphatase conjugate.

In practice this microsequencing analysis is performed as follows. 20 µl of the microsequencing reaction is added to 80 µl of capture buffer (SSC 2X, 2.5% PEG 8000, 0.25 M Tris pH7.5, 1.8% BSA, 0.05% Tween 20) and incubated for 20 minutes on a microtiter plate coated with streptavidin (Boehringer). The plate is rinsed once with washing buffer (0.1 M Tris pH 7.5, 0.1 M NaCl, 0.1% Tween 20). 100 µl of anti-fluorescein antibody conjugated with phosphatase alkaline, diluted 1/5000 in washing buffer containing 1.8% BSA is added to the microtiter plate. The antibody is incubated on the microtiter plate for 20 minutes. After washing the microtiter plate four times, 100 µl of 4-methylumbelliferyl phosphate (Sigma) diluted to 0.4 mg/ml in 0.1 M diethanolamine pH 9.6, 10mM $MgCl_2$ are added. The detection of the microsequencing reaction is carried out on a fluorimeter (Dynatech) after 20 minutes of incubation.

As another alternative, solid phase microsequencing reactions have been developed, for which either the oligonucleotide microsequencing primers or the PCR-amplified products derived from the DNA fragment of interest are immobilized. For example, immobilization can be carried out via an interaction between biotinylated DNA and streptavidin-coated microtitration wells or avidin-coated polystyrene particles.

As a further alternative, the PCR reaction generating the amplicons to be genotyped can be performed directly in solid phase conditions, following procedures such as those described in WO 96/13609.

In such solid phase microsequencing reactions, incorporated ddNTPs can either be radiolabeled (see Syvänen, *Clin. Chim. Acta.* 226:225-236 (1994)) or linked to fluorescein (see Livak and Hainer, *Hum. Metat.* 3:379-385 (1994)). The detection of radiolabeled ddNTPs can be achieved through scintillation-based techniques. The detection of fluorescein-linked ddNTPs can be based on the binding of antifluorescein antibody conjugated with alkaline phosphatase, followed by incubation with a chromogenic substrate (such as p-nitrophenyl phosphate).

Other possible reporter-detection couples for use in the above microsequencing procedures include:

-ddNTP linked to dinitrophenyl (DNP) and anti-DNP alkaline phosphatase conjugate (see Harju et al., *Clin Chem*:39(11Pt 1):2282-2287 (1993))

-biotinylated ddNTP and horseradish peroxidase-conjugated streptavidin with o-phenylenediamine as a substrate (see WO 92/15712).

A diagnosis kit based on fluorescein-linked ddNTP with antifluorescein antibody conjugated with alkaline phosphatase has been commercialized under the name PRONTO by GamidaGen Ltd.

As yet another alternative microsequencing procedure, Nyren et al. (*Anal. Biochem.* 208:171-175 (1993)) have described a solid-phase DNA sequencing procedure that relies on the detection of DNA polymerase activity by an enzymatic luminometric inorganic pyrophosphate detection assay (ELIDA). In this procedure, the PCR-amplified products are biotinylated and immobilized on beads. The microsequencing primer is annealed and four aliquots of this mixture are separately incubated with DNA polymerase and one of the four different ddNTPs. After the reaction, the resulting fragments are washed and used as substrates in a primer extension reaction with all four dNTPs present. The progress of the DNA-directed polymerization reactions is monitored with the ELIDA. Incorporation of a ddNTP in the first reaction prevents the formation of pyrophosphate during the subsequent dNTP reaction. In contrast, no ddNTP incorporation in the first reaction gives extensive pyrophosphate release during the dNTP reaction and this leads to generation of light throughout the ELIDA reactions. From the ELIDA results, the identity of the first base after the primer is easily deduced.

It will be appreciated that several parameters of the above-described microsequencing procedures may be successfully modified by those skilled in the art without undue experimentation. In particular, high throughput improvements to these procedures may be elaborated, following principles such as those described further below.

Example 9

Sequence Analysis

DNA sequences, such as BAC inserts, containing the region carrying the candidate gene associated with the detectable trait are sequenced and their sequence is analyzed using automated software which eliminates repeat sequences while retaining potential gene sequences. The potential gene sequences are compared to numerous databases to identify potential exons using a set of scoring algorithms such as trained Hidden Markov Models, statistical analysis models (including promoter prediction tools) and the GRAIL neural network. Preferred databases for use in this analysis, the construction and use of which are further detailed in Example 17, include the following:

NRPU (Non-Redundant Protein-Unique) database: NRPU is a non-redundant merge of the publicly available NBRF/PIR, Genpept, and SwissProt databases. Homologies found with NRPU allow the identification of regions potentially coding for already known proteins or related to known proteins (translated exons).

NREST (Non-Redundant EST database): NREST is a merge of the EST subsection of the publicly available GenBank database. Homologies found with NREST allow the location of potentially transcribed regions (translated or non-translated exons).

NRN (Non-Redundant Nucleic acid database): NRN is a merge of GenBank, EMBL and their daily updates.

Any sequence giving a positive hit with NRPU, NREST or an "excellent" score using GRAIL or/and other scoring algorithms is considered a potential functional region, and is then considered a candidate for genomic analysis.

While this first screening allows the detection of the "strongest" exons, a semi-automatic scan is further applied to the remaining sequences in the context of the sequence assembly. That is, the sequences neighboring a 5' site or an exon are submitted to another round of bioinformatics analysis with modified parameters. In this way, new exon candidates are generated for genomic analysis.

Using the above procedures, genes associated with detectable traits may be identified.

Example 10

YAC Contig Construction in the Candidate Genomic Region

Substantial amounts of LOH data supported the hypothesis that genes associated with distinct cancer types are located within a particular region of the human genome. More specifically, this region was likely to harbor a gene associated with prostate cancer.

Association studies were performed as described below in order to identify this prostate cancer gene. First, a YAC contig which contains the candidate genomic region was constructed as follows. The CEPH-Genethon YAC map for the entire human genome (Chumakov et al. (1995), *supra*) was used for detailed contig building in the genomic region containing genetic markers known to map in the candidate genomic region. Screening data available for several publicly available genetic markers were used to select a set of CEPH YACs localized within the candidate region. This set of YACs was tested by PCR with the above mentioned genetic markers as well as with other publicly available markers supposedly located within the candidate region. As a result of these studies, a YAC STS contig map was generated around genetic markers known to map in this genomic region. Two CEPH YACs were found to constitute a minimal tiling path in this region, with an estimated size of ca. 2 Megabases.

During this mapping effort, several publicly known STS markers were precisely located within the contig.

Example 11 below describes the identification of sets of biallelic markers within the candidate genomic region.

Example 11

BAC contig construction and

Biallelic Markers isolation within the candidate chromosomal region.

Next, a BAC contig covering the candidate genomic region was constructed as follows. BAC libraries were obtained as described in Woo et al., *Nucleic Acids Res.* 22:4922-4931 (1994). Briefly, the two whole human genome BamHI and HindIII libraries already described in related WIPO application No. PCT/IB98/00193 were constructed using the pBeloBAC11 vector (Kim et al. (1996), *supra*).

The BAC libraries were then screened with all of the above mentioned STSs, following the procedure described in Example 1 above.

The ordered BACs selected by STS screening and verified by FISH, were assembled into contigs and new markers were generated by partial sequencing of insert ends from some of them. These markers were used to fill the gaps in the contig of BAC clones covering the candidate chromosomal region having an estimated size of 2 megabases.

Figure 9 illustrates a minimal array of overlapping clones which was chosen for further studies, and the positions of the publicly known STS markers along said contig.

Selected BAC clones from the contig were subcloned and sequenced, essentially following the procedures described in related WIPO application No. PCT/IB98/00193.

Biallelic markers lying along the contig were identified following the processes described in related WIPO application No. PCT/IB98/00193.

Figure 9 shows the locations of the biallelic markers along the BAC contig. This first set of markers corresponds to a medium density map of the candidate locus, with an inter-marker distance averaging 50kb-150kb.

A second set of biallelic markers was then generated as described above in order to provide a very high-density map of the region identified using the first set of markers which can be used to conduct association studies, as explained below. This very high density map has markers spaced on average every 2-50kb.

The biallelic markers were then used in association studies. DNA samples were obtained from individuals suffering from prostate cancer and unaffected individuals as described in Example 12.

Example 12

Collection of DNA Samples from Affected and Non-affected Individuals

Prostate cancer patients were recruited according to clinical inclusion criteria based on pathological or radical prostatectomy records. Control cases included in this study were both ethnically- and age-matched to the affected cases; they were checked for both the absence of all clinical and biological criteria defining the presence or the risk of prostate

cancer, and for the absence of related familial prostate cancer cases. Both affected and control individuals were all unrelated.

The two following groups of independent individuals were used in the association studies. The first group, comprising individuals suffering from prostate cancer, contained 185 individuals. Of these 185 cases of prostate cancer, 47 cases were sporadic and 138 cases were familial. The control group contained 104 non-diseased individuals.

Haplotype analysis was conducted using additional diseased (total samples: 281) and control samples (total samples: 130), from individuals recruited according to similar criteria.

DNA was extracted from peripheral venous blood of all individuals as described in related WIPO application No. PCT/IB98/00193.

The frequencies of the biallelic markers in each population were determined as described in Example 13.

Example 13

Genotyping Affected and Control Individuals

Genotyping was performed using the following microsequencing procedure. Amplification was performed on each DNA sample using primers designed as previously explained. The pairs of primers of SEQ ID Nos.: 7849 to 7860 and 11780 to 11791 were used to generate amplicons harboring the biallelic markers of SEQ ID Nos: 3915 to 3926 or the sequences complementary thereto (markers 99-123-381, 4-26-29, 4-14-240, 4-77-151, 99-217-277, 4-67-40, 99-213-164, 99-221-377, 99-135-196, 99-1482-32, 4-73-134, and 4-65-324) using the protocols described in related WIPO application No. PCT/IB98/00193.

Microsequencing primers were designed for each of the biallelic markers, as previously described. After purification of the amplification products, the microsequencing reaction mixture was prepared by adding, in a 20µl final volume: 10 pmol microsequencing oligonucleotide, 1 U Thermosequenase (Amersham E79000G), 1.25 µl Thermosequenase buffer (260 mM Tris HCl pH 9.5, 65 mM $MgCl_2$), and the two appropriate fluorescent ddNTPs (Perkin Elmer, Dye Terminator Set 401095) complementary to the nucleotides at the polymorphic site of each biallelic marker tested, following the manufacturer's recommendations. After 4 minutes at 94°C, 20 PCR cycles of 15 sec at 55°C, 5 sec at 72°C, and 10 sec at 94°C were carried out in a Tetrad PTC-225 thermocycler (MJ Research). The unincorporated dye terminators were then removed by ethanol precipitation. Samples were finally resuspended in formamide-EDTA loading buffer and heated for 2 min at 95°C before being loaded on a polyacrylamide sequencing gel. The data were collected by an ABI PRISM 377 DNA sequencer and processed using the GENESCAN software (Perkin Elmer).

Following gel analysis, data were automatically processed with software that allows the determination of the alleles of biallelic markers present in each amplified fragment.

The software evaluates such factors as whether the intensities of the signals resulting from the above microsequencing procedures are weak, normal, or saturated, or whether the signals are ambiguous. In addition, the software identifies significant peaks (according to shape and height criteria). Among the significant peaks, peaks corresponding to the targeted site are identified based on their position. When two significant peaks are detected for the same position, each sample is categorized as homozygous or heterozygous based on the height ratio.

Association analyzes were then performed using the biallelic markers as described below.

Example 14

Association Analysis

Association studies were run in two successive steps. In a first step, a rough localization of the candidate gene was achieved by determining the frequencies of the biallelic markers of Figure 9 in the affected and unaffected populations. The results of this rough localization are shown in Figure 10. This analysis indicated that a gene responsible for prostate cancer was located near the biallelic marker designated 4-67.

In a second phase of the analysis, the position of the gene responsible for prostate cancer was further refined using the very high density set of markers including the markers of SEQ ID Nos: 3915 to 3926 or the sequences complementary thereto (markers 99-123-381, 4-26-29, 4-14-240, 4-77-151, 99-217-277, 4-67-40, 99-213-164, 99-221-377, 99-135-196, 99-1482-32, 4-73-134, and 4-65-324) .

As shown in Figure 11, the second phase of the analysis confirmed that the gene responsible for prostate cancer was near the biallelic marker designated 4-67-40, most probably within a ca. 150kb region comprising the marker.

A haplotype analysis was also performed as described in Example 15.

Example 15

Haplotype analysis

The allelic frequencies of each of the alleles of biallelic markers 99-123-381, 4-26-29, 4-14-240, 4-77-151, 99-217-277, 4-67-40, 99-213-164, 99-221-377, and 99-135-196 were determined in the affected and unaffected populations. Table 4 lists the internal identification numbers of the markers used in the haplotype analysis (SEQ ID Nos: 3915-3923), the alleles of each marker, the most frequent allele in both unaffected individuals and individuals suffering from prostate cancer, the least frequent allele in both unaffected individuals and individuals suffering from prostate cancer, and the frequencies of the least frequent alleles in each population.

Table 4

Markers	Polymorphic base *	Frequency of least frequent allele ** Cases	Controls
99-123-381	C/T	0.35	0.3
4-26-29	A/G	0.39	0.45
4-14-240	C/T	0.35	0.41
4-77-151	C/G	0.33	0.24
99-217-277	C/T	0.31	0.23
4-67-40	C/T	0.26	0.16
99-213-164	T/C	0.45	0.38
99-221-377	C/A	0.43	0.43
99-135-196	A/G	0.25	0.3

*most frequent allele/least frequent allele
**standard deviations - 0.023 to 0.031 for controls
- 0.018 to 0.021 for cases

Among all the theoretical potential different haplotypes based on 2 to 9 markers, 11 haplotypes showing a strong association with prostate cancer were selected. The results of these haplotype analyzes are shown in Figure 12.

Figures 11 and 12 aggregate association analysis results with sequencing results – generated following the procedures further described in Example 16, which permitted the physical order and the distance between markers to be estimated.

The significance of the values obtained in Figure 12 are underscored by the following results of computer simulations. For the computer simulations, the data from the affected individuals and the unaffected controls were pooled and randomly allocated to two groups which contained the same number of individuals as the affected and unaffected groups used to compile the data summarized in Figure 12. A haplotype analysis was run on these artificial groups for the six markers included in haplotype 5 of Figure 12. This experiment was reiterated 100 times and the results are shown in Figure 13. Among 100 iterations, only 5% of the obtained haplotypes are present with a p-value less significant than E-04 as compared to the p-value of 9E-07 for haplotype 5 of Figure 12. Furthermore, for haplotype 5 of Figure 12, only 6% of the obtained haplotypes have a significance level below 5^{E}-03, while none of them show a significance level below 5E-03.

Thus, using the data of Figure 13 and evaluating the associations for single marker alleles or for haplotypes will permit estimation of the risk a corresponding carrier has to develop prostate cancer. It will be appreciated that significance thresholds of relative risks will be more finely assessed according to the population tested.

Diagnostic techniques for determining an individual's risk of developing prostate cancer may be implemented as described below for the markers in the maps of the present invention, including the markers of SEQ ID Nos: 3915 to 3923 (markers 99-123-381, 4-26-29, 4-14-240, 4-77-151, 99-217-277, 4-67-40, 99-213-164, 99-221-377, and 99-135-196).

The above haplotype analysis indicated that 171kb of genomic DNA between biallelic markers 4-14-240 and 99-221-377 totally or partially contains a gene responsible for prostate cancer. Therefore, the protein coding sequences lying within this region were characterized to locate the gene associated with prostate cancer. This analysis, described in further detail below, revealed a single protein coding sequence in the 171 kb genomic region, which was designated as the PG1 gene.

Example 16

Identification of the Genomic Sequence in the Candidate Region

Template DNA for sequencing the PG1 gene was obtained as follows. BACs E and F from Fig. 9 were subcloned as previously described. Plasmid inserts were first amplified by PCR on PE 9600 thermocyclers (Perkin-Elmer), using appropriate primers, AmpliTaqGold (Perkin-Elmer), dNTPs (Boehringer), buffer and cycling conditions as recommended by the Perkin-Elmer Corporation.

PCR products were then sequenced using automatic ABI Prism 377 sequencers (Perkin Elmer, Applied Biosystems Division, Foster City, CA). Sequencing reactions were performed using PE 9600 thermocyclers (Perkin Elmer) with standard dye-primer chemistry and ThermoSequenase (Amersham Life Science). The primers were labeled with the JOE, FAM, ROX and TAMRA dyes. The dNTPs and ddNTPs used in the sequencing reactions were purchased from Boehringer. Sequencing buffer, reagent concentrations and cycling conditions were as recommended by Amersham.

Following the sequencing reaction, the samples were precipitated with EtOH, resuspended in formamide loading buffer, and loaded on a standard 4% acrylamide gel. Electrophoresis was performed for 2.5 hours at 3000V on an ABI 377 sequencer, and the sequence data were collected and analyzed using the ABI Prism DNA Sequencing Analysis Software, version 2.1.2.

The sequence data obtained as described above were transferred to a proprietary database, where quality control and validation steps were performed. A proprietary base-caller flagged suspect peaks, taking into account the shape of the peaks, the inter-peak resolution, and the noise level. The proprietary base-caller also performed an automatic trimming. Any stretch of 25 or fewer bases having more than 4 suspect peaks was considered unreliable and was discarded.

The sequence fragments from BAC subclones isolated as described above were assembled using Gap4 software from R. Staden (Bonfield et al. 1995). This software allows the reconstruction of a single sequence from sequence fragments. The sequence deduced from the alignment of different fragments is called the consensus sequence. Directed sequencing techniques (primer walking) were used to complete sequences and link contigs.

Potential functional sequences were then identified as described in Example 17.

Example 17

Identification of Functional Sequences

Potential exons in BAC-derived human genomic sequences were located by homology searches on protein, nucleic acid and EST (Expressed Sequence Tags) public databases. Main public databases were locally reconstructed as mentioned in Example 9. The protein database, NRPU (Non-redundant Protein Unique) is formed by a non-redundant fusion of the Genpept (Benson et al., *Nucleic Acids Res.* 24:1-5 (1996)), Swissprot (Bairoch, A. and Apweiler, R., *Nucleic Acids Res.* 24:21-25 (1996)) and PIR/NBRF (George et al., *Nucleic Acids Res.* 24:17-20 (1996)) databases. Redundant data were eliminated by using the NRDB software (Benson et al. (1996), *supra*) and internal repeats were masked with the XNU software (Benson et al., *supra*). Homologies found using the NRPU database allowed the identification of sequences corresponding to potential coding exons related to known proteins.

The EST local database is composed by the gbest section (1-9) of GenBank (Benson et al. (1996), *supra*), and thus contains all publicly available transcript fragments. Homologies found with this database allowed the localization of potentially transcribed regions.

The local nucleic acid database contained all sections of GenBank and EMBL (Rodriguez-Tome et al., *Nucleic Acids Res.* 24:6-12 (1996)) except the EST sections. Redundant data were eliminated as previously described.

Similarity searches in protein or nucleic acid databases were performed using the BLAST software (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990)). Alignments were refined using the Fasta software, and multiple alignments used Clustal W. Homology thresholds were adjusted for each analysis based on the length and the complexity of the tested region, as well as on the size of the reference database.

Potential exon sequences identified as above were used as probes to screen cDNA libraries. Extremities of positive clones were sequenced and the sequence stretches were positioned on the genomic sequence determined above. Primers were then designed using the results from these alignments in order to enable the cloning of cDNAs derived from the gene associated with prostate cancer that was identified using the above procedures.

The obtained cDNA molecules were then sequenced and results of Northern blot analysis of prostate mRNAs supported the existence of a major cDNA having a 5-6kb length.

The structure of the gene associated with prostate cancer was evaluated as described in Example 18.

Example 18

Analysis of Gene Structure

The intron/exon structure of the gene was finally completely deduced by aligning the mRNA sequence from the cDNA obtained as described above and the genomic DNA sequence obtained as described above. This alignment permitted the determination of the positions of the introns and exons, the positions of the start and end nucleotides defining each of the at least 8 exons, the locations and phases of the 5' and 3' splice sites, the position of the stop codon, and the position of the polyadenylation site to be determined in the genomic sequence. This analysis also yielded the positions of the coding region in the mRNA, and the locations of the polyadenylation signal and polyA stretch in the mRNA.

The gene identified as described above comprises at least 8 exons and spans more than 52kb. A G/C rich putative promoter region was identified upstream of the coding sequence. A CCAAT in the putative promoter was also identified. The promoter region was identified as described in Prestridge, D.S., Predicting Pol II Promoter Sequences Using Transcription Factor Binding Sites, *J. Mol. Biol.* 249:923-932 (1995).

Additional analysis using conventional techniques, such as a 5'RACE reaction using the Marathon-Ready human prostate cDNA kit from Clontech (Catalog. No. PT1156-1), may be performed to confirm that the 5' of the cDNA obtained above is the authentic 5' end in the mRNA.

Alternatively, the 5'sequence of the transcript can be determined by conducting a PCR amplification with a series of primers extending from the 5'end of the identified coding region.

Example 19

Detection of biallelic markers in the candidate gene: DNA extraction

Donors were unrelated and healthy. They presented a sufficient diversity for being representative of a French heterogeneous population. The DNA from 100 individuals was extracted and tested for the detection of the biallelic markers.

30 ml of peripheral venous blood were taken from each donor in the presence of EDTA. Cells (pellet) were collected after centrifugation for 10 minutes at 2000 rpm. Red cells were lysed by a lysis solution (50 ml final volume: 10 mM Tris pH7.6; 5 mM MgCl2; 10 mM NaCl). The solution was centrifuged (10 minutes, 2000 rpm) as many times as necessary to eliminate the residual red cells present in the supernatant, after resuspension of the pellet in the lysis solution.

The pellet of white cells was lysed overnight at 42°C with 3.7 ml of lysis solution composed of:

- 3 ml TE 10-2 (Tris-HCl 10 mM, EDTA 2 mM) / NaCl 0.4 M
- 200 µl SDS 10%
- 500 µl K-proteinase (2 mg K-proteinase in TE 10-2 / NaCl 0.4 M).

For the extraction of proteins, 1 ml saturated NaCl (6M) (1/3.5 v/v) was added. After vigorous agitation, the solution was centrifuged for 20 minutes at 10000 rpm.

For the precipitation of DNA, 2 to 3 volumes of 100% ethanol were added to the previous supernatant, and the solution was centrifuged for 30 minutes at 2000 rpm. The DNA solution was rinsed three times with 70% ethanol to eliminate salts, and centrifuged for 20 minutes at 2000 rpm. The pellet was dried at 37°C, and resuspended in 1 ml TE 10-1 or 1 ml water. The DNA concentration was evaluated by measuring the OD at 260 nm (1 unit OD = 50 µg/ml DNA).

To determine the presence of proteins in the DNA solution, the OD 260 / OD 280 ratio was determined. Only DNA preparations having a OD 260 / OD 280 ratio between 1.8 and 2 were used in the subsequent examples described below.

The pool was constituted by mixing equivalent quantities of DNA from each individual.

Example 20

Detection of the biallelic markers: amplification of genomic DNA by PCR

The amplification of specific genomic sequences of the DNA samples of Example 19 was carried out on the pool of DNA obtained previously using the amplification primers of SEQ ID Nos: 7861 to 7865 and 11792 to 11796. In addition, 50 individual samples were similarly amplified.

PCR assays were performed using the following protocol:

Final volume	25 µl
DNA	2 ng/µl
MgCl2	2 mM
dNTP (each)	200 µM
primer (each)	2.9 ng/µl
Ampli Taq Gold DNA polymerase	0.05 unit/µl
PCR buffer (10x = 0.1 M TrisHCl pH8.3 0.5M KCl)	1x

Pairs of first primers were designed to amplify the promoter region, exons, and 3' end of the candidate asthma-associated gene using the sequence information of the candidate gene

and the OSP software (Hillier & Green, 1991). These first primers were about 20 nucleotides in length and contained a common oligonucleotide tail upstream of the specific bases targeted for amplification which was useful for sequencing. The synthesis of these primers was performed following the phosphoramidite method, on a GENSET UFPS 24.1 synthesizer.

DNA amplification was performed on a Genius II thermocycler. After heating at 94°C for 10 min, 40 cycles were performed. Each cycle comprised: 30 sec at 94°C, 55°C for 1 min, and 30 sec at 72°C. For final elongation, 7 min at 72°C ended the amplification. The quantities of the amplification products obtained were determined on 96-well microtiter plates, using a fluorometer and Picogreen as intercalant agent (Molecular Probes).

Example 21

Detection of the biallelic markers

Sequencing of amplified genomic DNA and identification of polymorphisms

The sequencing of the amplified DNA obtained in Example 20 was carried out on ABI 377 sequencers. The sequences of the amplification products were determined using automated dideoxy terminator sequencing reactions with a dye terminator cycle sequencing protocol. The products of the sequencing reactions were run on sequencing gels and the sequences were analyzed as formerly described.

The sequence data were further evaluated using the above mentioned polymorphism analysis software designed to detect the presence of biallelic markers among the pooled amplified fragments. The polymorphism search was based on the presence of superimposed peaks in the electrophoresis pattern resulting from different bases occurring at the same position as described previously.

Six fragments of amplification were analyzed. In these segments, 8 biallelic markers were detected (SEQ ID Nos: 3927 to 3934). The localization of the biallelic markers, the polymorphic bases of each allele, and the frequencies of the most frequent alleles was as shown in Table 5.

Table 5

Amplicon	Marker Name	Origin of DNA	Localization in gene	Polymorphism	Frequency
1	10-204-326	Ind.	Promoter	A/G	96.2 (G)
2	10-32-357	Pool	Intron 1	A/C	67.7 (C)
3	10-33-175	Ind.	Exon 2	C/T	97.3 (C)
3	10-33-234	Pool	Intron 2	A/C	56.7 (C)
3	10-33-327	Ind.	Intron 2	C/T	75.3 (T)

5	10-35-358	Pool	Intron 4	C/G	67.9 (G)
5	10-35-390	Ind.	Intron 4	C/T	82 (C)
6	10-36-164	Ind.	Exon 5	A/G	99.5 (G)

Allelic frequencies were determined in a population of random blood donors from French Caucasian origin. Their wide range is due to the fact that, besides screening a pool of 100 individuals to generate biallelic markers as described above, polymorphism searches were also conducted in an individual testing format for 50 samples. This strategy was chosen here to provide a potential shortcut towards the identification of putative causal mutations in the association studies using them. As the 10-36-164 biallelic marker (SEQ ID No: 3933) was found in only one individual, this marker was not considered in the association studies.

The fourth fragment of amplification carrying exon 3 (not shown in the Table) was not polymorphic in the tested samples (1 pool + 50 individuals).

Example 22

Validation of the polymorphisms through microsequencing

The biallelic markers identified in Example 21 were further confirmed and their respective frequencies were determined through microsequencing. Microsequencing was carried out for each individual DNA sample described in Example 19.

Amplification from genomic DNA of individuals was performed by PCR as described above for the detection of the biallelic markers with the same set of PCR primers described above.

The preferred primers used in microsequencing had about 19 nucleotides in length and hybridized just upstream of the considered polymorphic base.

Five primers hybridized with the non-coding strand of the gene. For the biallelic markers 10-204-326, 10-35-358 and 10-36-164, primers hybridized with the coding strand of the gene.

The microsequencing reaction was performed as described in Example 13.

Example 23

Association study between asthma and the biallelic markers of the candidate gene

Collection of DNA samples from affected and non-affected individuals

The asthmatic population used to perform association studies in order to establish whether the candidate gene was an asthma-causing gene consisted of 298 individuals. More than 90 % of these 298 asthmatic individuals had a Caucasian ethnic background.

The control population consisted of 373 unaffected individuals, among which 279 French (at least 70 % were of Caucasian origin) and 94 American (at least 90 % were of Caucasian origin).

DNA samples were obtained from asthmatic and non-asthmatic individuals as described above.

Example 24

Association study between asthma and the biallelic markers of the candidate gene

Genotyping of affected and control individuals

The general strategy to perform the association studies was to individually scan the DNA samples from all individuals in each of the populations described above in order to establish the allele frequencies of the above described biallelic markers in each of these populations.

Allelic frequencies of the above-described biallelic markers in each population were determined by performing microsequencing reactions on amplified fragments obtained by genomic PCR performed on the DNA samples from each individual. Genomic PCR and microsequencing were performed as detailed above in Examples 20 and 22 using the described amplification and microsequencing primers.

Example 25

Association study between asthma and the biallelic markers of the candidate gene

Table 6 shows the results of the association study between five biallelic markers in the candidate gene and asthma.

Table 6

Allelic frequencies (%)				
Markers	**Asthmatics 298 individuals**	**Controls 373 individuals**	**Frequency diff.**	**P value**
10-32-357	A 38.6	A 29.8	8.8	**$7.34x10^{-4}$**
10-33-234	A 49	A 44.3	4.7	$8.86x10^{-2}$
10-33-327	T 78.5	T 74.6	3.9	$1.0x10^{-1}$
10-35-358	G 72.3	G 66.9	5.4	$3.59x10^{-2}$
10-35-390	T 30.4	T 20.3	10.1	**$2.33x10^{-5}$**

As shown in Table 6, markers 10-32-357 and 10-35-390 presented a strong association with asthma, this association being highly significant (pvalue = 7.34x10-4 for marker 10-32-357 and 2.33x10-5 for marker 10-35-390).

Three markers showed moderate association when tested independently, namely 10-33-234, 10-33-327,10- 35-358.

It is worth mentioning that allelic frequencies for each of the biallelic markers of Table 7 were separately measured within the French control population (279 individuals) and the American control population (94 individuals). The differences in allele frequencies

between the two populations were between 1% and 7%, with p-values above 10^{-1}. These data confirmed that the combined French/American control population (373 individuals) was homogeneous enough to be used as a control population for the present association study.

Example 26

Association studies: Haplotype frequency analysis

As already shown, one way of increasing the statistical power of individual markers, is by performing haplotype association analysis. A haplotype analysis for association of markers in the candidate gene and asthma was performed by estimating the frequencies of all possible haplotypes for biallelic markers 10-32-357, 10-33-234, 10-33-327, 10-35-358 and 10-35-390 in the asthmatic and control populations described in Example 25 (Table 6), and comparing these frequencies by means of a chi square statistical test (one degree of freedom). Haplotype estimations were performed by applying the Expectation-Maximization (EM) algorithm (Excoffier L & Slatkin M, 1995, Mol.Biol.Evol. 12 :921-927), using the EM-HAPLO program (Hawley ME, Pakstis AJ & Kidd KK, 1994, Am.J.Phys.Anthropol. 18:104).

The results of such haplotype analysis are shown in Table 7.

Table 7

					Haplotype frequencies			
Markers	**10-32-357**	**10-33-234**	**10-33-327**	**10-35-358**	**10-35-390**	**Asthm.**	**Controls**	**Odds ratio**	**P value**
Frequency diff.	8.8	4.7	3.9	5.4	10.1				
P value	7.34×10^{-4}	8.86×10^{-2}	1.0×10^{-1}	3.59×10^{-2}	2.33×10^{-5}				
Haplotype 1	**A**	**T**	0.2	0.11	2.02	**8.47×10^{-6}**			
Haplotype 2	**A**	**T**	**G**	0.27	0.18	1.68	**2.81×10^{-4}**		
Haplotype 3	**A**	**A**	**T**	**G**	**T**	0.18	0.09	2.22	3.95×10^{-5}

A two-marker haplotype covering markers 10-32-357 and 10-35-390 (haplotype 1, AT alleles respectively) presented a p value of 8.47x10-6, an odds ratio of 2.02 and haplotype frequencies of 0.2 for asthmatic and 0.11 for control populations respectively.

A three-marker haplotype covering markers 10-33-234, 10-33-327 and 10-35-358 (haplotype 2, ATG alleles respectively) presented a p value of 2.81x10-4, an odds ratio of 1.68 and haplotype frequencies of 0.27 for asthmatic and 0.18 for control populations respectively.

A five-marker haplotype covering markers 10-32-357, 10-33-234, 10-33-327, 10-35-358 and 10-35-390 (haplotype 3, AATGT alleles respectively) presented a p value of 3.95x10-5, an odds ratio of 2.22 and haplotype frequencies of 0.18 for asthmatic and 0.09 for control populations respectively.

Haplotype association analysis thus increased the statistical power of the individual marker association studies when compared to single-marker analysis (from p values between 10^{-1} and $2X10^{-5}$ for the individual markers to p values between $3X10^{-4}$ and $8X10^{-6}$ for the three-marker haplotype, haplotype 2).

The significance of the values obtained for the haplotype association analysis was evaluated by the following computer simulation test. The genotype data from the asthmatic and control individuals were pooled and randomly allocated to two groups which contained the same number of individuals as the trait-positive and trait-negative groups used to produce the data summarized in Table 7. A haplotype analysis was then run on these artificial groups for the three haplotypes presented in Table 6. This experiment was reiterated 1000 times and the results are shown in Table 8.

Table 8

Haplotype	Chi-Square Average	Chi-Square	Permutation Test Maximal Chi-Square	Permutation Test P value
Haplotype 1 (A—T)	19.70	1.2	11.6	$1.0x10^{-3}$
Haplotype 2 (-ATG-)	13.49	1.2	10.5	$1.0x10^{-3}$
Haplotype 3 (AATGT)	16.66	1.2	9.3	$1.0x10^{-3}$

The results in Table 8 show that among 1000 iterations only 1‰ of the obtained haplotypes has a pvalue comparable to the one obtained in Table 4.

These results clearly validate the statistical significance of the haplotypes obtained (haplotypes 1, 2 and 3, Table 7).

Example 27

Extraction of DNA

30 ml of blood are taken from the individuals in the presence of EDTA. Cells (pellet) are collected after centrifugation for 10 minutes at 2000 rpm. Red cells are lysed by a lysis solution (50 ml final volume : 10 mM Tris pH7.6; 5 mM $MgCl_2$; 10 mM NaCl). The solution is centrifuged (10 minutes, 2000 rpm) as many times as necessary to eliminate the residual red cells present in the supernatant, after resuspension of the pellet in the lysis solution.

The pellet of white cells is lysed overnight at 42°C with 3.7 ml of lysis solution composed of:

- 3 ml TE 10-2 (Tris-HCl 10 mM, EDTA 2 mM) / NaCl 0.4 M
- 200 µl SDS 10%

- 500 µl K-proteinase (2 mg K-proteinase in TE 10-2 / NaCl 0.4 M).

For the extraction of proteins, 1 ml saturated NaCl (6M) (1/3.5 v/v) is added. After vigorous agitation, the solution is centrifuged for 20 minutes at 10000 rpm.

For the precipitation of DNA, 2 to 3 volumes of 100% ethanol are added to the previous supernatant, and the solution is centrifuged for 30 minutes at 2000 rpm. The DNA solution is rinsed three times with 70% ethanol to eliminate salts, and centrifuged for 20 minutes at 2000 rpm. The pellet is dried at 37°C, and resuspended in 1 ml TE 10-1 or 1 ml water. The DNA concentration is evaluated by measuring the OD at 260 nm (1 unit OD = 50 µg/ml DNA).

To evaluate the presence of proteins in the DNA solution, the OD 260 / OD 280 ratio is determined. Only DNA preparations having a OD 260 / OD 280 ratio between 1.8 and 2 are used in the subsequent steps described below.

Once genomic DNA from every individual in the given population has been extracted, it is preferred that a fraction of each DNA sample is separated, after which a pool of DNA is constituted by assembling equivalent DNA amounts of the separated fractions into a single one.

TABLE 1

SEQ ID No.	Marker Name	Allele		Preferred microseq. primer	Amplification primer	
		1ST	2ND		Upstream (PU)	Downstream (RP)
1	99-109-224	G	C	S	3935	7866
2	99-1126-384	A	G	S	3936	7867
3	99-114-68	G	C	S	3937	7868
4	99-1151-516	A	C	S	3938	7869
5	99-1165-159	C	T	S	3939	7870
6	99-1167-201	A	G	A	3940	7871
7	99-117-205	C	T	S	3941	7872
8	99-118-92	C	T	S	3942	7873
9	99-1217-332	C	T	A	3943	7874
10	99-1233-183	A	G	S	3944	7875
11	99-12478-263	G	T	A	3945	7876
12	99-12487-301	A	C	S	3946	7877
13	99-12497-155	C	T	S	3947	7878
14	99-12503-44	G	C	S	3948	7879
15	99-12504-402	A	T	S	3949	7880
16	99-12505-374	A	G	A	3950	7881
17	99-12506-199	G	T	A	3951	7882
18	99-12509-423	C	T	S	3952	7883
19	99-12513-146	G	C	S	3953	7884
20	99-12514-170	G	C	S	3954	7885
21	99-12515-205	G	C	S	3955	7886
22	99-12516-524	A	G	A	3956	7887
23	99-12518-325	C	T	S	3957	7888
24	99-12523-255	C	T	S	3958	7889
25	99-12525-277	C	T	S	3959	7890
26	99-12526-317	C	T	S	3960	7891
27	99-12527-292	A	G	A	3961	7892
28	99-12531-30	C	T	S	3962	7893
29	99-12532-199	A	T	S	3963	7894
30	99-12534-207	A	C	S	3964	7895
31	99-12535-362	A	C	S	3965	7896
32	99-12537-340	G	C	S	3966	7897
33	99-12538-142	A	C	S	3967	7898
34	99-12539-287	C	T	S	3968	7899
35	99-12540-426	C	T	S	3969	7900
36	99-12541-307	C	T	S	3970	7901
37	99-12545-121	A	G	A	3971	7902
38	99-12548-88	A	G	A	3972	7903
39	99-12558-167	C	T	S	3973	7904
40	99-12562-291	C	T	S	3974	7905
41	99-12564-354	A	T	S	3975	7906
42	99-12565-273	C	T	S	3976	7907
43	99-12575-248	G	C	S	3977	7908
44	99-12576-325	C	T	S	3978	7909
45	99-12580-268	A	G	A	3979	7910
46	99-12585-85	A	C	S	3980	7911
47	99-12593-103	A	C	S	3981	7912
48	99-12600-283	G	C	S	3982	7913
49	99-12608-71	C	T	S	3983	7914

50	99-12610-106	C	T	S	3984	7915
51	99-12611-311	G	T	A	3985	7916
52	99-12613-366	A	G	A	3986	7917
53	99-12615-235	A	C	S	3987	7918
54	99-12617-412	G	C	S	3988	7919
55	99-12618-211	C	T	S	3989	7920
56	99-12619-367	A	G	A	3990	7921
57	99-12621-114	A	G	A	3991	7922
58	99-12624-61	A	T	S	3992	7923
59	99-1263-276	A	G	S	3993	7924
60	99-12632-165	C	T	S	3994	7925
61	99-12637-62	C	T	S	3995	7926
62	99-12639-311	G	C	S	3996	7927
63	99-12640-179	C	T	A	3997	7928
64	99-12650-200	C	T	A	3998	7929
65	99-12651-297	G	C	S	3999	7930
66	99-12652-459	A	G	S	4000	7931
67	99-12654-278	G	T	A	4001	7932
68	99-12656-303	C	T	A	4002	7933
69	99-12658-206	C	T	A	4003	7934
70	99-12661-92	G	T	A	4004	7935
71	99-12668-329	C	T	A	4005	7936
72	99-1268-177	A	G	A	4006	7937
73	99-12733-366	G	C	S	4007	7938
74	99-12738-57	G	C	S	4008	7939
75	99-12740-354	C	T	A	4009	7940
76	99-12749-286	A	G	S	4010	7941
77	99-12750-369	A	T	S	4011	7942
78	99-12751-406	C	T	A	4012	7943
79	99-12755-421	A	G	S	4013	7944
80	99-12756-344	A	C	S	4014	7945
81	99-12757-240	A	G	S	4015	7946
82	99-12759-420	G	T	A	4016	7947
83	99-12777-71	A	G	S	4017	7948
84	99-12782-76	A	C	S	4018	7949
85	99-12794-299	G	C	S	4019	7950
86	99-128-60	C	T	S	4020	7951
87	99-12816-101	G	C	S	4021	7952
88	99-12817-358	C	T	A	4022	7953
89	99-12819-165	A	G	S	4023	7954
90	99-12826-408	A	T	S	4024	7955
91	99-12831-345	A	C	S	4025	7956
92	99-12836-387	C	T	A	4026	7957
93	99-12842-305	C	T	A	4027	7958
94	99-12843-337	A	G	S	4028	7959
95	99-12844-130	A	G	S	4029	7960
96	99-12847-37	A	G	S	4030	7961
97	99-12848-204	A	G	S	4031	7962
98	99-12852-260	A	G	S	4032	7963
99	99-12856-183	A	C	S	4033	7964
100	99-12878-291	C	T	S	4034	7965
101	99-12880-282	C	T	S	4035	7966
102	99-12884-248	A	G	A	4036	7967
103	99-12885-261	A	C	S	4037	7968
104	99-12898-364	C	T	S	4038	7969
105	99-12899-307	C	T	S	4039	7970

106	99-1290-291	C	T	S	4040	7971
107	99-12900-165	G	C	S	4041	7972
108	99-12901-316	A	G	A	4042	7973
109	99-12903-381	C	T	S	4043	7974
110	99-12907-295	A	G	A	4044	7975
111	99-12908-369	G	C	S	4045	7976
112	99-12913-197	C	T	S	4046	7977
113	99-12914-227	G	T	A	4047	7978
114	99-12924-273	G	C	S	4048	7979
115	99-12925-487	C	T	S	4049	7980
116	99-12926-332	C	T	A	4050	7981
117	99-12931-173	A	G	S	4051	7982
118	99-12948-61	A	T	S	4052	7983
119	99-12952-199	G	C	S	4053	7984
120	99-12956-43	C	T	A	4054	7985
121	99-12957-448	C	T	A	4055	7986
122	99-12961-318	A	G	S	4056	7987
123	99-12962-181	A	G	S	4057	7988
124	99-12963-255	C	T	A	4058	7989
125	99-12964-230	C	T	A	4059	7990
126	99-13021-124	C	T	S	4060	7991
127	99-13036-313	A	C	S	4061	7992
128	99-13045-385	A	C	S	4062	7993
129	99-13051-235	A	G	S	4063	7994
130	99-13061-100	C	T	S	4064	7995
131	99-13064-328	C	T	S	4065	7996
132	99-13065-311	C	T	S	4066	7997
133	99-13070-207	G	T	A	4067	7998
134	99-13098-369	A	G	S	4068	7999
135	99-13106-251	A	G	S	4069	8000
136	99-13115-106	C	T	A	4070	8001
137	99-13121-198	A	G	S	4071	8002
138	99-13130-75	A	G	S	4072	8003
139	99-13133-341	A	G	S	4073	8004
140	99-13134-79	A	G	S	4074	8005
141	99-13165-216	A	G	S	4075	8006
142	99-13178-252	C	T	A	4076	8007
143	99-13192-272	A	G	S	4077	8008
144	99-13193-453	C	T	A	4078	8009
145	99-13201-154	C	T	A	4079	8010
146	99-13203-79	A	G	S	4080	8011
147	99-13215-109	C	T	A	4081	8012
148	99-13218-103	G	C	S	4082	8013
149	99-13219-378	A	G	S	4083	8014
150	99-13222-274	C	T	A	4084	8015
151	99-13224-351	C	T	S	4085	8016
152	99-13227-270	A	C	S	4086	8017
153	99-13229-192	G	T	A	4087	8018
154	99-13232-494	G	C	S	4088	8019
155	99-13237-44	G	T	S	4089	8020
156	99-13238-276	A	G	S	4090	8021
157	99-13241-49	C	T	A	4091	8022
158	99-13246-251	A	G	S	4092	8023
159	99-13250-439	C	T	A	4093	8024
160	99-13251-118	G	T	A	4094	8025
161	99-13258-232	G	T	A	4095	8026

162	99-13260-358	G	C	S	4096	8027
163	99-13262-376	G	C	S	4097	8028
164	99-13269-144	A	G	S	4098	8029
165	99-13270-309	G	T	A	4099	8030
166	99-13271-163	A	G	S	4100	8031
167	99-13272-151	A	G	S	4101	8032
168	99-13273-144	A	C	S	4102	8033
169	99-13276-168	C	T	A	4103	8034
170	99-13279-301	A	G	S	4104	8035
171	99-13286-58	A	G	S	4105	8036
172	99-13287-298	C	T	A	4106	8037
173	99-13294-281	A	G	S	4107	8038
174	99-13296-330	C	T	A	4108	8039
175	99-13320-352	A	G	S	4109	8040
176	99-13332-259	C	T	A	4110	8041
177	99-13334-136	A	C	S	4111	8042
178	99-13336-364	A	G	S	4112	8043
179	99-13339-335	G	C	S	4113	8044
180	99-13354-225	A	G	S	4114	8045
181	99-13368-221	C	T	A	4115	8046
182	99-13394-42	A	G	S	4116	8047
183	99-13395-110	C	T	A	4117	8048
184	99-13396-258	G	C	S	4118	8049
185	99-134-362	G	T	A	4119	8050
186	99-13401-106	A	C	A	4120	8051
187	99-13404-373	A	G	S	4121	8052
188	99-13406-279	G	T	A	4122	8053
189	99-1342-51	C	T	A	4123	8054
190	99-13429-188	A	C	S	4124	8055
191	99-13439-327	A	G	S	4125	8056
192	99-13443-275	C	T	A	4126	8057
193	99-13450-276	A	G	S	4127	8058
194	99-13457-138	G	C	S	4128	8059
195	99-1346-503	C	T	S	4129	8060
196	99-13462-263	A	G	S	4130	8061
197	99-13486-358	A	G	S	4131	8062
198	99-13489-396	C	T	A	4132	8063
199	99-13499-445	A	G	S	4133	8064
200	99-13502-118	C	T	S	4134	8065
201	99-13509-388	A	G	A	4135	8066
202	99-1351-264	A	T	S	4136	8067
203	99-13515-428	C	T	S	4137	8068
204	99-13525-395	C	T	S	4138	8069
205	99-13526-368	C	T	S	4139	8070
206	99-13531-449	A	T	S	4140	8071
207	99-13536-134	C	T	S	4141	8072
208	99-13540-338	G	C	S	4142	8073
209	99-13541-85	A	G	A	4143	8074
210	99-13545-215	C	T	S	4144	8075
211	99-13552-172	A	C	S	4145	8076
212	99-13553-390	A	C	S	4146	8077
213	99-13555-402	A	G	A	4147	8078
214	99-1356-500	A	T	S	4148	8079
215	99-13567-258	C	T	S	4149	8080
216	99-13586-230	G	T	A	4150	8081
217	99-13588-238	A	C	S	4151	8082

218	99-13589-362	A	G	A	4152	8083
219	99-1359-355	C	T	S	4153	8084
220	99-13591-360	G	C	S	4154	8085
221	99-13592-304	A	C	S	4155	8086
222	99-13596-69	A	C	S	4156	8087
223	99-13598-260	G	C	S	4157	8088
224	99-13600-305	A	G	S	4158	8089
225	99-13601-360	A	G	S	4159	8090
226	99-13605-208	C	T	A	4160	8091
227	99-13606-83	G	C	S	4161	8092
228	99-1362-126	A	G	A	4162	8093
229	99-13624-415	C	T	A	4163	8094
230	99-13638-354	A	G	S	4164	8095
231	99-13644-439	G	C	S	4165	8096
232	99-13647-278	C	T	A	4166	8097
233	99-13652-407	G	C	S	4167	8098
234	99-13663-218	C	T	A	4168	8099
235	99-13666-275	A	T	S	4169	8100
236	99-1367-287	A	G	A	4170	8101
237	99-13671-396	C	T	A	4171	8102
238	99-13678-251	C	T	A	4172	8103
239	99-13679-285	C	T	A	4173	8104
240	99-1368-299	C	T	S	4174	8105
241	99-13684-488	A	C	S	4175	8106
242	99-13687-316	A	G	S	4176	8107
243	99-1373-358	A	T	S	4177	8108
244	99-1376-196	A	T	S	4178	8109
245	99-13790-129	C	T	A	4179	8110
246	99-13798-284	A	G	S	4180	8111
247	99-13831-102	A	G	S	4181	8112
248	99-13832-226	C	T	A	4182	8113
249	99-13835-39	G	C	S	4183	8114
250	99-1385-91	G	C	S	4184	8115
251	99-13853-256	C	T	A	4185	8116
252	99-13854-363	C	T	A	4186	8117
253	99-13860-368	C	T	A	4187	8118
254	99-13861-227	A	G	S	4188	8119
255	99-13866-198	C	T	A	4189	8120
256	99-13868-240	C	T	A	4190	8121
257	99-1387-462	C	T	A	4191	8122
258	99-13876-55	A	G	S	4192	8123
259	99-13878-385	A	C	S	4193	8124
260	99-1388-242	A	G	A	4194	8125
261	99-13880-185	A	G	S	4195	8126
262	99-13883-103	A	G	S	4196	8127
263	99-13887-190	C	T	A	4197	8128
264	99-13888-332	C	T	A	4198	8129
265	99-13892-338	A	G	S	4199	8130
266	99-13897-431	G	T	A	4200	8131
267	99-1391-204	C	T	S	4201	8132
268	99-13912-89	C	T	A	4202	8133
269	99-13913-278	A	G	S	4203	8134
270	99-13914-169	A	G	S	4204	8135
271	99-1392-200	C	T	S	4205	8136
272	99-13920-172	A	G	S	4206	8137
273	99-13925-97	A	G	S	4207	8138

274	99-13929-201	A	C	S	4208	8139
275	99-13932-229	C	T	A	4209	8140
276	99-1394-271	A	G	A	4210	8141
277	99-13956-119	G	C	S	4211	8142
278	99-13960-142	C	T	A	4212	8143
279	99-13962-339	A	G	S	4213	8144
280	99-13980-150	C	T	A	4214	8145
281	99-13996-123	A	G	S	4215	8146
282	99-13997-181	C	T	A	4216	8147
283	99-13998-421	C	T	A	4217	8148
284	99-140-130	C	T	S	4218	8149
285	99-14004-328	G	C	S	4219	8150
286	99-14005-344	C	T	A	4220	8151
287	99-14009-133	C	T	A	4221	8152
288	99-14010-165	C	T	A	4222	8153
289	99-14013-125	C	T	A	4223	8154
290	99-14025-459	A	G	S	4224	8155
291	99-1404-135	A	G	S	4225	8156
292	99-14046-270	A	G	S	4226	8157
293	99-14050-295	G	T	A	4227	8158
294	99-14068-214	C	T	A	4228	8159
295	99-14072-363	C	T	A	4229	8160
296	99-14080-436	G	T	A	4230	8161
297	99-14083-346	A	G	S	4231	8162
298	99-14087-429	G	T	A	4232	8163
299	99-14090-398	A	T	S	4233	8164
300	99-14094-274	A	T	S	4234	8165
301	99-14119-101	C	T	S	4235	8166
302	99-14120-283	A	G	A	4236	8167
303	99-14127-127	A	G	A	4237	8168
304	99-1413-137	G	C	S	4238	8169
305	99-14135-375	C	T	S	4239	8170
306	99-14139-321	A	T	S	4240	8171
307	99-14140-310	A	G	A	4241	8172
308	99-14145-220	G	C	S	4242	8173
309	99-14147-369	A	G	A	4243	8174
310	99-14149-351	G	C	S	4244	8175
311	99-1416-589	A	G	A	4245	8176
312	99-14161-267	A	G	A	4246	8177
313	99-14162-180	C	T	S	4247	8178
314	99-14166-217	G	C	S	4248	8179
315	99-14175-380	A	G	S	4249	8180
316	99-14179-191	A	G	S	4250	8181
317	99-14186-424	A	G	S	4251	8182
318	99-14197-144	A	G	S	4252	8183
319	99-14203-268	A	C	S	4253	8184
320	99-14204-468	G	T	A	4254	8185
321	99-14220-351	A	G	S	4255	8186
322	99-1423-361	A	C	S	4256	8187
323	99-14250-381	A	G	S	4257	8188
324	99-14254-305	G	T	A	4258	8189
325	99-14256-133	C	T	A	4259	8190
326	99-1426-185	C	T	S	4260	8191
327	99-14260-261	C	T	A	4261	8192
328	99-14277-73	A	G	S	4262	8193
329	99-14282-334	A	C	S	4263	8194

330	99-14285-381	C	T	A	4264	8195
331	99-14286-220	G	T	A	4265	8196
332	99-14309-259	C	T	S	4266	8197
333	99-14315-405	A	C	S	4267	8198
334	99-14329-205	G	C	S	4268	8199
335	99-14331-64	A	G	S	4269	8200
336	99-14332-437	C	T	A	4270	8201
337	99-14343-408	A	G	S	4271	8202
338	99-14345-139	C	T	A	4272	8203
339	99-14356-141	A	G	S	4273	8204
340	99-1437-325	C	T	S	4274	8205
341	99-14385-117	A	T	S	4275	8206
342	99-14392-431	A	C	S	4276	8207
343	99-14393-190	C	T	A	4277	8208
344	99-144-392	C	T	S	4278	8209
345	99-14405-105	A	G	S	4279	8210
346	99-1442-224	G	T	A	4280	8211
347	99-14444-193	G	C	S	4281	8212
348	99-14446-337	A	G	S	4282	8213
349	99-14452-263	C	T	S	4283	8214
350	99-14459-44	G	C	S	4284	8215
351	99-14468-247	C	T	A	4285	8216
352	99-14470-243	A	G	S	4286	8217
353	99-14492-322	C	T	A	4287	8218
354	99-14497-220	A	G	S	4288	8219
355	99-14505-250	C	T	A	4289	8220
356	99-14518-57	C	T	A	4290	8221
357	99-1453-204	C	T	S	4291	8222
358	99-14553-224	C	T	S	4292	8223
359	99-14562-402	A	G	S	4293	8224
360	99-14566-320	C	T	A	4294	8225
361	99-14574-310	G	C	S	4295	8226
362	99-14581-365	C	T	A	4296	8227
363	99-14591-172	G	C	S	4297	8228
364	99-14595-210	C	T	A	4298	8229
365	99-14596-174	C	T	A	4299	8230
366	99-14597-85	G	C	S	4300	8231
367	99-14598-91	C	T	A	4301	8232
368	99-14599-220	C	T	A	4302	8233
369	99-14600-207	A	G	S	4303	8234
370	99-14601-448	C	T	A	4304	8235
371	99-14607-267	C	T	A	4305	8236
372	99-14609-467	G	T	A	4306	8237
373	99-14610-351	A	C	S	4307	8238
374	99-14611-241	G	C	S	4308	8239
375	99-14612-100	G	C	S	4309	8240
376	99-14614-248	A	G	S	4310	8241
377	99-14615-65	A	G	S	4311	8242
378	99-14616-35	A	G	S	4312	8243
379	99-14618-147	G	C	S	4313	8244
380	99-14619-325	A	C	S	4314	8245
381	99-1462-238	G	C	S	4315	8246
382	99-14620-253	C	T	A	4316	8247
383	99-14621-96	G	C	S	4317	8248
384	99-14622-276	C	T	A	4318	8249
385	99-14626-307	A	G	S	4319	8250

386	99-14627-272	C	T	A	4320	8251
387	99-14628-312	A	C	S	4321	8252
388	99-14629-274	A	G	S	4322	8253
389	99-14630-75	C	T	A	4323	8254
390	99-14634-350	A	G	S	4324	8255
391	99-14635-296	G	C	S	4325	8256
392	99-14637-366	G	C	S	4326	8257
393	99-14638-276	A	G	S	4327	8258
394	99-14643-27	C	T	S	4328	8259
395	99-14644-395	G	C	S	4329	8260
396	99-14647-227	C	T	A	4330	8261
397	99-14651-205	A	G	S	4331	8262
398	99-14652-120	A	G	S	4332	8263
399	99-14653-138	C	T	A	4333	8264
400	99-14662-352	A	G	S	4334	8265
401	99-14664-289	C	T	A	4335	8266
402	99-14665-199	A	G	S	4336	8267
403	99-14669-238	A	G	S	4337	8268
404	99-14671-175	C	T	A	4338	8269
405	99-14676-313	A	T	S	4339	8270
406	99-14677-358	G	C	S	4340	8271
407	99-14678-75	G	C	S	4341	8272
408	99-14679-241	C	T	A	4342	8273
409	99-1468-435	C	T	S	4343	8274
410	99-1469-47	G	C	S	4344	8275
411	99-14690-84	G	T	A	4345	8276
412	99-14692-46	A	G	S	4346	8277
413	99-14699-149	C	T	A	4347	8278
414	99-147-181	A	G	A	4348	8279
415	99-14701-264	A	G	S	4349	8280
416	99-14704-59	A	C	S	4350	8281
417	99-14708-142	G	T	A	4351	8282
418	99-1471-571	C	T	S	4352	8283
419	99-14710-107	C	T	A	4353	8284
420	99-14712-163	C	T	A	4354	8285
421	99-14714-237	C	T	S	4355	8286
422	99-14717-132	A	G	S	4356	8287
423	99-1472-435	A	G	A	4357	8288
424	99-14722-272	C	T	A	4358	8289
425	99-14729-284	A	T	S	4359	8290
426	99-14733-26	A	T	S	4360	8291
427	99-14735-328	A	G	S	4361	8292
428	99-1474-156	G	T	A	4362	8293
429	99-14746-377	A	G	S	4363	8294
430	99-14753-194	G	T	A	4364	8295
431	99-14756-270	A	T	S	4365	8296
432	99-1476-172	G	C	S	4366	8297
433	99-14761-194	A	G	S	4367	8298
434	99-14773-383	G	T	A	4368	8299
435	99-14776-79	G	C	S	4369	8300
436	99-14777-100	C	T	S	4370	8301
437	99-14782-152	G	C	S	4371	8302
438	99-14784-212	A	G	S	4372	8303
439	99-14785-92	A	C	S	4373	8304
440	99-14786-59	A	G	S	4374	8305
441	99-1479-158	C	T	S	4375	8306

442	99-14792-43	C	T	S	4376	8307
443	99-14796-227	C	T	A	4377	8308
444	99-14799-57	A	G	S	4378	8309
445	99-148-182	A	G	A	4379	8310
446	99-1480-290	G	T	A	4380	8311
447	99-14802-60	A	T	S	4381	8312
448	99-14803-157	C	T	A	4382	8313
449	99-14804-216	A	G	S	4383	8314
450	99-14805-58	A	G	S	4384	8315
451	99-14806-108	G	C	S	4385	8316
452	99-14807-150	G	C	S	4386	8317
453	99-1481-285	G	T	A	4387	8318
454	99-14810-407	C	T	A	4388	8319
455	99-14812-189	G	C	S	4389	8320
456	99-14817-323	C	T	A	4390	8321
457	99-14818-430	A	G	S	4391	8322
458	99-14819-278	G	C	S	4392	8323
459	99-14820-76	A	T	S	4393	8324
460	99-14821-48	C	T	A	4394	8325
461	99-14826-238	G	T	A	4395	8326
462	99-14828-214	C	T	A	4396	8327
463	99-14833-226	A	T	S	4397	8328
464	99-1484-328	G	C	S	4398	8329
465	99-14843-195	G	T	A	4399	8330
466	99-14844-143	C	T	A	4400	8331
467	99-1485-251	G	T	A	4401	8332
468	99-14850-136	A	G	S	4402	8333
469	99-14856-260	A	G	S	4403	8334
470	99-14861-387	A	G	S	4404	8335
471	99-14862-171	A	T	S	4405	8336
472	99-14865-386	A	G	S	4406	8337
473	99-14867-160	A	T	S	4407	8338
474	99-14872-326	A	G	S	4408	8339
475	99-14873-453	C	T	A	4409	8340
476	99-14875-411	C	T	A	4410	8341
477	99-14879-398	G	C	S	4411	8342
478	99-14881-231	C	T	A	4412	8343
479	99-14882-382	G	C	S	4413	8344
480	99-14883-123	G	C	S	4414	8345
481	99-1489-76	A	C	S	4415	8346
482	99-14890-358	G	T	A	4416	8347
483	99-14892-237	A	G	S	4417	8348
484	99-14894-52	A	G	S	4418	8349
485	99-14895-343	C	T	A	4419	8350
486	99-14897-356	C	T	A	4420	8351
487	99-1490-381	C	T	S	4421	8352
488	99-14907-411	G	T	A	4422	8353
489	99-1493-280	A	G	A	4423	8354
490	99-14937-42	A	C	S	4424	8355
491	99-14939-240	A	C	S	4425	8356
492	99-1494-598	A	G	A	4426	8357
493	99-14940-224	A	G	S	4427	8358
494	99-14950-346	A	T	S	4428	8359
495	99-14959-81	A	T	S	4429	8360
496	99-14961-193	G	C	S	4430	8361
497	99-14962-120	A	G	S	4431	8362

498	99-14966-187	A	G	S	4432	8363
499	99-14970-352	A	G	S	4433	8364
500	99-14978-200	G	T	A	4434	8365
501	99-1498-120	C	T	S	4435	8366
502	99-14983-186	G	T	A	4436	8367
503	99-14984-35	C	T	A	4437	8368
504	99-15005-169	C	T	A	4438	8369
505	99-15007-369	A	G	S	4439	8370
506	99-1501-296	A	G	A	4440	8371
507	99-15016-293	C	T	A	4441	8372
508	99-15018-270	A	G	S	4442	8373
509	99-15019-408	A	G	S	4443	8374
510	99-15021-189	A	G	S	4444	8375
511	99-15030-271	A	C	S	4445	8376
512	99-15039-277	G	T	A	4446	8377
513	99-1504-252	A	G	A	4447	8378
514	99-15043-175	A	G	S	4448	8379
515	99-15046-54	C	T	A	4449	8380
516	99-1506-505	C	T	S	4450	8381
517	99-15072-64	C	T	A	4451	8382
518	99-15087-77	C	T	A	4452	8383
519	99-15098-367	C	T	A	4453	8384
520	99-151-94	A	G	A	4454	8385
521	99-15100-363	C	T	A	4455	8386
522	99-15101-154	G	C	S	4456	8387
523	99-15106-451	C	T	A	4457	8388
524	99-15107-228	A	G	S	4458	8389
525	99-15112-358	C	T	A	4459	8390
526	99-15118-69	A	G	S	4460	8391
527	99-15123-180	C	T	A	4461	8392
528	99-15128-349	C	T	A	4462	8393
529	99-15129-279	C	T	A	4463	8394
530	99-15135-231	A	G	S	4464	8395
531	99-15137-386	A	G	S	4465	8396
532	99-1515-402	A	G	A	4466	8397
533	99-15160-270	A	G	S	4467	8398
534	99-15164-67	C	T	A	4468	8399
535	99-15193-143	G	T	A	4469	8400
536	99-15195-377	A	C	S	4470	8401
537	99-15199-179	C	T	A	4471	8402
538	99-1520-143	C	T	S	4472	8403
539	99-15200-196	A	G	S	4473	8404
540	99-15202-357	G	C	S	4474	8405
541	99-1521-457	G	C	S	4475	8406
542	99-1525-102	C	T	A	4476	8407
543	99-15290-343	G	T	A	4477	8408
544	99-15296-326	G	C	S	4478	8409
545	99-15302-371	G	T	A	4479	8410
546	99-15307-251	C	T	A	4480	8411
547	99-15310-385	A	G	S	4481	8412
548	99-15325-95	A	G	A	4482	8413
549	99-15328-328	C	T	A	4483	8414
550	99-1533-471	G	T	A	4484	8415
551	99-15330-301	G	C	S	4485	8416
552	99-15335-313	A	G	S	4486	8417
553	99-15339-378	C	T	A	4487	8418

554	99-15345-376	G	T	A	4488	8419
555	99-1535-241	C	T	S	4489	8420
556	99-1537-243	G	T	A	4490	8421
557	99-15374-99	A	G	S	4491	8422
558	99-15377-206	A	G	S	4492	8423
559	99-15382-388	G	T	A	4493	8424
560	99-15393-177	A	T	S	4494	8425
561	99-15406-220	A	C	S	4495	8426
562	99-15425-132	C	T	A	4496	8427
563	99-15441-337	A	C	S	4497	8428
564	99-15446-339	A	G	S	4498	8429
565	99-15457-171	A	C	S	4499	8430
566	99-15458-308	C	T	A	4500	8431
567	99-15473-339	A	C	S	4501	8432
568	99-15486-309	A	C	S	4502	8433
569	99-15489-305	G	C	S	4503	8434
570	99-1549-124	A	G	A	4504	8435
571	99-15490-398	A	C	S	4505	8436
572	99-15493-197	A	G	S	4506	8437
573	99-15500-77	A	G	S	4507	8438
574	99-15502-250	G	C	S	4508	8439
575	99-15503-85	G	T	A	4509	8440
576	99-15507-248	C	T	A	4510	8441
577	99-15508-259	C	T	A	4511	8442
578	99-15511-278	A	G	S	4512	8443
579	99-15516-155	A	G	S	4513	8444
580	99-15524-224	C	T	A	4514	8445
581	99-15526-324	A	T	S	4515	8446
582	99-15527-154	A	G	S	4516	8447
583	99-15528-333	A	G	S	4517	8448
584	99-1553-544	A	C	S	4518	8449
585	99-15543-55	C	T	A	4519	8450
586	99-15545-282	A	G	S	4520	8451
587	99-15557-50	A	G	S	4521	8452
588	99-1557-251	C	T	S	4522	8453
589	99-15574-261	G	T	A	4523	8454
590	99-15575-278	G	T	A	4524	8455
591	99-1558-26	C	T	S	4525	8456
592	99-15595-41	A	G	A	4526	8457
593	99-15596-64	C	T	S	4527	8458
594	99-15599-252	A	G	S	4528	8459
595	99-15605-221	A	G	S	4529	8460
596	99-15606-326	C	T	A	4530	8461
597	99-15625-299	G	C	S	4531	8462
598	99-15627-324	A	C	S	4532	8463
599	99-15636-159	A	G	S	4533	8464
600	99-15638-65	C	T	A	4534	8465
601	99-15648-83	A	G	S	4535	8466
602	99-15659-332	G	C	S	4536	8467
603	99-1568-240	A	T	S	4537	8468
604	99-15705-110	C	T	S	4538	8469
605	99-15717-120	C	T	A	4539	8470
606	99-15718-234	C	T	A	4540	8471
607	99-1572-440	C	T	S	4541	8472
608	99-15728-334	A	T	S	4542	8473
609	99-15739-113	C	T	A	4543	8474

610	99-15744-344	C	T	A	4544	8475
611	99-15747-185	C	T	A	4545	8476
612	99-15748-360	A	C	S	4546	8477
613	99-15756-54	C	T	A	4547	8478
614	99-15758-119	C	T	A	4548	8479
615	99-15762-455	A	G	S	4549	8480
616	99-1577-105	A	G	A	4550	8481
617	99-15774-268	A	G	S	4551	8482
618	99-15776-158	C	T	A	4552	8483
619	99-1578-496	C	T	S	4553	8484
620	99-15798-86	C	T	A	4554	8485
621	99-15803-52	A	G	S	4555	8486
622	99-15805-327	A	G	S	4556	8487
623	99-1582-430	C	T	S	4557	8488
624	99-15826-407	G	T	A	4558	8489
625	99-15830-282	A	G	S	4559	8490
626	99-1585-373	C	T	S	4560	8491
627	99-1587-281	A	G	A	4561	8492
628	99-15891-215	A	G	S	4562	8493
629	99-15910-116	A	C	S	4563	8494
630	99-15916-270	A	G	S	4564	8495
631	99-15925-331	C	T	A	4565	8496
632	99-15947-109	C	T	A	4566	8497
633	99-15965-67	A	G	S	4567	8498
634	99-15966-87	C	T	A	4568	8499
635	99-15968-59	A	G	S	4569	8500
636	99-1597-162	A	G	A	4570	8501
637	99-15970-56	A	G	S	4571	8502
638	99-15973-73	C	T	A	4572	8503
639	99-15981-298	A	G	S	4573	8504
640	99-15985-354	A	G	S	4574	8505
641	99-15992-145	A	C	S	4575	8506
642	99-15996-361	G	T	A	4576	8507
643	99-16003-91	G	C	S	4577	8508
644	99-16005-314	C	T	A	4578	8509
645	99-1601-402	A	T	S	4579	8510
646	99-1602-200	G	C	S	4580	8511
647	99-16022-325	A	G	S	4581	8512
648	99-16023-160	G	C	S	4582	8513
649	99-16030-317	A	G	S	4583	8514
650	99-1605-112	A	G	A	4584	8515
651	99-1607-373	A	G	A	4585	8516
652	99-1611-382	A	G	A	4586	8517
653	99-16121-51	C	T	A	4587	8518
654	99-16128-88	C	T	A	4588	8519
655	99-16129-69	C	T	A	4589	8520
656	99-16139-79	A	G	S	4590	8521
657	99-16140-324	A	G	S	4591	8522
658	99-1615-118	A	G	A	4592	8523
659	99-16166-331	A	G	S	4593	8524
660	99-16167-130	G	C	S	4594	8525
661	99-16188-76	A	G	S	4595	8526
662	99-16192-217	A	C	S	4596	8527
663	99-16198-203	A	T	S	4597	8528
664	99-16202-240	A	G	S	4598	8529
665	99-16205-255	A	G	S	4599	8530

666	99-16210-217	A	T	S	4600	8531
667	99-1622-158	G	C	S	4601	8532
668	99-16221-161	G	C	S	4602	8533
669	99-16227-270	A	G	S	4603	8534
670	99-1623-145	C	T	S	4604	8535
671	99-16247-447	A	G	S	4605	8536
672	99-16254-304	A	G	S	4606	8537
673	99-16260-277	A	G	S	4607	8538
674	99-16262-232	C	T	A	4608	8539
675	99-16265-88	G	C	S	4609	8540
676	99-16279-409	G	T	A	4610	8541
677	99-16308-315	A	G	S	4611	8542
678	99-16346-433	C	T	A	4612	8543
679	99-16366-425	A	T	S	4613	8544
680	99-1637-345	C	T	S	4614	8545
681	99-16375-469	C	T	A	4615	8546
682	99-1638-571	C	T	S	4616	8547
683	99-16386-484	G	C	S	4617	8548
684	99-16396-174	C	T	A	4618	8549
685	99-16399-135	A	C	S	4619	8550
686	99-16403-273	A	G	S	4620	8551
687	99-16407-260	A	C	S	4621	8552
688	99-16409-58	A	T	S	4622	8553
689	99-16414-297	A	C	S	4623	8554
690	99-16445-444	C	T	A	4624	8555
691	99-16466-419	A	C	S	4625	8556
692	99-16474-299	G	T	A	4626	8557
693	99-16500-380	A	T	S	4627	8558
694	99-16505-368	G	C	S	4628	8559
695	99-16526-375	A	C	S	4629	8560
696	99-16528-194	C	T	A	4630	8561
697	99-16531-251	A	G	S	4631	8562
698	99-16535-344	C	T	A	4632	8563
699	99-16559-90	A	G	S	4633	8564
700	99-16562-182	A	G	S	4634	8565
701	99-16563-263	G	C	S	4635	8566
702	99-16564-118	A	G	S	4636	8567
703	99-16568-283	G	T	A	4637	8568
704	99-16569-65	A	G	S	4638	8569
705	99-1658-474	C	T	S	4639	8570
706	99-16611-318	A	C	S	4640	8571
707	99-1664-289	C	T	S	4641	8572
708	99-16655-135	C	T	S	4642	8573
709	99-16657-361	A	G	S	4643	8574
710	99-16677-346	C	T	S	4644	8575
711	99-16683-465	A	C	S	4645	8576
712	99-16692-343	G	T	A	4646	8577
713	99-16697-149	A	C	S	4647	8578
714	99-16708-273	A	G	A	4648	8579
715	99-16740-391	G	T	A	4649	8580
716	99-16768-54	A	T	S	4650	8581
717	99-16801-374	C	T	S	4651	8582
718	99-16827-356	A	G	A	4652	8583
719	99-16838-212	C	T	S	4653	8584
720	99-16841-120	A	T	S	4654	8585
721	99-16842-362	A	G	A	4655	8586

722	99-16845-234	A	G	A	4656	8587
723	99-16847-405	A	G	A	4657	8588
724	99-16855-84	G	C	S	4658	8589
725	99-16867-193	C	T	S	4659	8590
726	99-16873-407	G	C	S	4660	8591
727	99-16886-198	G	T	A	4661	8592
728	99-16891-264	C	T	A	4662	8593
729	99-16894-85	C	T	A	4663	8594
730	99-16895-56	A	C	S	4664	8595
731	99-16903-53	A	G	S	4665	8596
732	99-16905-281	C	T	A	4666	8597
733	99-16906-114	A	G	S	4667	8598
734	99-16913-94	A	G	S	4668	8599
735	99-16914-412	A	G	S	4669	8600
736	99-16929-479	A	C	S	4670	8601
737	99-16930-306	C	T	A	4671	8602
738	99-16933-33	A	G	S	4672	8603
739	99-16946-157	A	C	S	4673	8604
740	99-16948-390	A	T	S	4674	8605
741	99-16952-248	A	C	S	4675	8606
742	99-16975-253	G	C	S	4676	8607
743	99-16979-274	A	G	S	4677	8608
744	99-16990-155	C	T	A	4678	8609
745	99-16994-63	A	G	S	4679	8610
746	99-17001-311	C	T	A	4680	8611
747	99-17008-420	C	T	S	4681	8612
748	99-1701-39	A	G	A	4682	8613
749	99-17013-217	G	T	A	4683	8614
750	99-17016-258	A	G	A	4684	8615
751	99-17028-56	A	G	A	4685	8616
752	99-17045-267	A	G	A	4686	8617
753	99-17048-207	A	C	S	4687	8618
754	99-17052-71	A	G	A	4688	8619
755	99-17062-102	A	G	S	4689	8620
756	99-17065-230	A	G	S	4690	8621
757	99-17084-191	A	G	S	4691	8622
758	99-1709-597	G	C	S	4692	8623
759	99-17094-132	C	T	S	4693	8624
760	99-17095-424	A	G	S	4694	8625
761	99-1710-249	G	C	S	4695	8626
762	99-17103-142	C	T	A	4696	8627
763	99-17105-147	A	G	S	4697	8628
764	99-17112-191	C	T	A	4698	8629
765	99-17122-255	G	C	S	4699	8630
766	99-17123-320	C	T	A	4700	8631
767	99-17133-327	A	T	S	4701	8632
768	99-17134-82	C	T	A	4702	8633
769	99-17136-384	A	G	S	4703	8634
770	99-17154-256	A	G	S	4704	8635
771	99-17157-33	A	G	S	4705	8636
772	99-17159-280	A	G	S	4706	8637
773	99-17164-252	G	C	S	4707	8638
774	99-17165-359	A	G	S	4708	8639
775	99-17169-485	A	T	S	4709	8640
776	99-17176-315	A	G	S	4710	8641
777	99-17180-309	A	G	S	4711	8642

778	99-17204-105	A	G	S	4712	8643
779	99-17205-68	C	T	A	4713	8644
780	99-17213-128	C	T	A	4714	8645
781	99-1723-101	A	G	A	4715	8646
782	99-17253-394	G	T	A	4716	8647
783	99-17262-65	G	T	A	4717	8648
784	99-17274-353	A	G	A	4718	8649
785	99-17282-138	A	T	S	4719	8650
786	99-17306-27	C	T	A	4720	8651
787	99-17315-86	A	T	S	4721	8652
788	99-17343-305	A	G	S	4722	8653
789	99-17347-160	C	T	A	4723	8654
790	99-17351-259	G	T	A	4724	8655
791	99-17352-284	C	T	A	4725	8656
792	99-17357-244	C	T	A	4726	8657
793	99-17363-245	A	G	S	4727	8658
794	99-17365-188	A	G	S	4728	8659
795	99-17375-363	G	C	S	4729	8660
796	99-1738-72	A	G	A	4730	8661
797	99-17389-164	G	T	A	4731	8662
798	99-1739-135	A	C	S	4732	8663
799	99-17409-293	C	T	S	4733	8664
800	99-17412-296	C	T	A	4734	8665
801	99-17416-310	A	G	S	4735	8666
802	99-17418-41	A	T	S	4736	8667
803	99-17420-380	A	G	S	4737	8668
804	99-17428-129	A	G	S	4738	8669
805	99-17450-352	A	G	S	4739	8670
806	99-17464-376	C	T	A	4740	8671
807	99-17476-141	A	C	S	4741	8672
808	99-17481-171	C	T	A	4742	8673
809	99-17483-282	G	T	A	4743	8674
810	99-17490-199	A	G	S	4744	8675
811	99-17491-362	C	T	A	4745	8676
812	99-17495-100	A	C	S	4746	8677
813	99-17496-301	A	C	S	4747	8678
814	99-17498-312	C	T	A	4748	8679
815	99-17499-62	A	G	S	4749	8680
816	99-17520-31	C	T	A	4750	8681
817	99-17522-423	A	G	S	4751	8682
818	99-17523-116	A	C	S	4752	8683
819	99-17529-210	A	G	S	4753	8684
820	99-17557-358	C	T	A	4754	8685
821	99-17563-102	G	C	S	4755	8686
822	99-17588-501	A	G	S	4756	8687
823	99-17610-44	A	G	S	4757	8688
824	99-17629-89	G	T	A	4758	8689
825	99-1764-65	G	C	S	4759	8690
826	99-17647-79	A	G	S	4760	8691
827	99-1765-171	C	T	S	4761	8692
828	99-17656-239	A	G	S	4762	8693
829	99-17658-167	A	G	S	4763	8694
830	99-17662-126	C	T	A	4764	8695
831	99-17663-29	A	G	S	4765	8696
832	99-17677-251	A	G	A	4766	8697
833	99-17680-451	C	T	S	4767	8698

834	99-17683-286	A	G	A	4768	8699
835	99-17687-373	C	T	S	4769	8700
836	99-17700-191	G	C	S	4770	8701
837	99-17702-57	G	T	A	4771	8702
838	99-17718-259	C	T	S	4772	8703
839	99-17720-224	G	C	S	4773	8704
840	99-17728-310	A	C	S	4774	8705
841	99-1773-343	C	T	S	4775	8706
842	99-17740-227	G	C	S	4776	8707
843	99-1775-187	A	G	A	4777	8708
844	99-17758-292	A	G	A	4778	8709
845	99-17762-327	A	G	A	4779	8710
846	99-17773-392	C	T	S	4780	8711
847	99-17774-276	C	T	S	4781	8712
848	99-17775-286	C	T	S	4782	8713
849	99-17776-114	A	T	S	4783	8714
850	99-17779-117	A	C	S	4784	8715
851	99-17792-144	C	T	S	4785	8716
852	99-17798-345	C	T	S	4786	8717
853	99-17802-338	A	G	A	4787	8718
854	99-17808-398	G	C	S	4788	8719
855	99-1781-129	A	G	A	4789	8720
856	99-17810-366	C	T	S	4790	8721
857	99-17816-377	G	C	S	4791	8722
858	99-17820-316	A	G	A	4792	8723
859	99-17821-109	A	G	S	4793	8724
860	99-17827-106	A	C	S	4794	8725
861	99-17829-412	G	C	S	4795	8726
862	99-17833-108	G	T	A	4796	8727
863	99-17845-286	A	G	A	4797	8728
864	99-17854-229	C	T	S	4798	8729
865	99-17856-308	G	C	S	4799	8730
866	99-17857-251	A	G	A	4800	8731
867	99-17863-257	A	G	A	4801	8732
868	99-17864-202	G	T	A	4802	8733
869	99-17866-124	C	T	S	4803	8734
870	99-17889-148	G	C	S	4804	8735
871	99-17890-58	C	T	S	4805	8736
872	99-17899-140	A	G	A	4806	8737
873	99-17913-222	G	T	A	4807	8738
874	99-17920-382	A	G	A	4808	8739
875	99-1793-225	A	T	S	4809	8740
876	99-17938-131	A	G	A	4810	8741
877	99-17945-63	A	T	S	4811	8742
878	99-17946-69	A	C	S	4812	8743
879	99-17952-370	C	T	S	4813	8744
880	99-1796-184	A	T	S	4814	8745
881	99-17971-78	A	G	A	4815	8746
882	99-17976-132	G	C	S	4816	8747
883	99-17989-85	A	G	A	4817	8748
884	99-17991-412	A	T	S	4818	8749
885	99-17992-404	A	G	A	4819	8750
886	99-18004-125	C	T	S	4820	8751
887	99-18007-159	C	T	S	4821	8752
888	99-18030-54	A	T	S	4822	8753
889	99-18038-384	G	C	S	4823	8754

890	99-18046-65	A	T	S	4824	8755
891	99-18053-328	A	G	S	4825	8756
892	99-18054-392	A	C	S	4826	8757
893	99-18056-354	A	G	S	4827	8758
894	99-18057-55	A	C	S	4828	8759
895	99-18060-203	G	T	A	4829	8760
896	99-18062-187	A	G	A	4830	8761
897	99-18069-282	C	T	A	4831	8762
898	99-18079-46	G	C	S	4832	8763
899	99-1808-291	A	T	S	4833	8764
900	99-18080-378	G	T	A	4834	8765
901	99-18085-94	A	G	S	4835	8766
902	99-18086-434	A	G	S	4836	8767
903	99-18087-152	C	T	A	4837	8768
904	99-18091-47	G	C	S	4838	8769
905	99-18096-198	C	T	A	4839	8770
906	99-18109-159	C	T	A	4840	8771
907	99-1813-310	C	T	S	4841	8772
908	99-18130-258	A	G	S	4842	8773
909	99-1814-245	A	G	A	4843	8774
910	99-18171-95	G	T	A	4844	8775
911	99-18172-284	A	G	S	4845	8776
912	99-18179-185	G	T	A	4846	8777
913	99-18198-203	C	T	A	4847	8778
914	99-18201-23	A	G	S	4848	8779
915	99-18206-76	A	G	S	4849	8780
916	99-18210-30	G	C	S	4850	8781
917	99-18213-185	A	G	S	4851	8782
918	99-18214-86	A	C	S	4852	8783
919	99-18221-207	C	T	A	4853	8784
920	99-1823-157	A	G	A	4854	8785
921	99-1824-226	A	G	A	4855	8786
922	99-18242-369	A	G	S	4856	8787
923	99-18253-407	C	T	S	4857	8788
924	99-18255-259	A	T	S	4858	8789
925	99-18258-45	G	C	S	4859	8790
926	99-18268-460	A	G	A	4860	8791
927	99-18272-287	G	C	S	4861	8792
928	99-18276-390	A	G	S	4862	8793
929	99-18288-205	A	G	A	4863	8794
930	99-18289-36	C	T	S	4864	8795
931	99-18303-79	C	T	S	4865	8796
932	99-18306-377	A	G	A	4866	8797
933	99-18307-371	A	C	S	4867	8798
934	99-18310-262	C	T	S	4868	8799
935	99-18312-58	C	T	S	4869	8800
936	99-18341-95	G	T	A	4870	8801
937	99-18344-284	A	G	A	4871	8802
938	99-18345-107	C	T	S	4872	8803
939	99-18371-433	A	T	S	4873	8804
940	99-18373-27	A	G	A	4874	8805
941	99-18375-237	A	G	A	4875	8806
942	99-18379-485	C	T	S	4876	8807
943	99-18386-177	A	T	S	4877	8808
944	99-18394-132	G	T	A	4878	8809
945	99-18402-255	C	T	S	4879	8810

946	99-18406-155	A	G	A	4880	8811
947	99-18414-204	A	C	S	4881	8812
948	99-18418-127	G	C	S	4882	8813
949	99-1842-78	C	T	S	4883	8814
950	99-18423-336	A	G	A	4884	8815
951	99-18427-314	C	T	S	4885	8816
952	99-18438-398	C	T	S	4886	8817
953	99-18442-283	C	T	S	4887	8818
954	99-18444-185	C	T	S	4888	8819
955	99-18458-191	A	G	A	4889	8820
956	99-18470-119	A	T	S	4890	8821
957	99-18478-101	A	G	A	4891	8822
958	99-18486-49	A	G	A	4892	8823
959	99-18487-236	C	T	S	4893	8824
960	99-18488-273	A	G	A	4894	8825
961	99-1849-421	C	T	S	4895	8826
962	99-18536-290	G	T	A	4896	8827
963	99-18542-232	A	C	S	4897	8828
964	99-18551-389	G	C	S	4898	8829
965	99-18561-371	A	C	S	4899	8830
966	99-18573-363	G	T	A	4900	8831
967	99-18582-422	A	G	A	4901	8832
968	99-18588-175	A	C	S	4902	8833
969	99-18596-83	A	G	A	4903	8834
970	99-18597-415	C	T	S	4904	8835
971	99-18599-347	A	G	A	4905	8836
972	99-1860-281	A	G	A	4906	8837
973	99-18602-241	A	G	A	4907	8838
974	99-18606-324	C	T	S	4908	8839
975	99-1861-191	G	C	S	4909	8840
976	99-18612-184	C	T	S	4910	8841
977	99-18618-455	C	T	S	4911	8842
978	99-18620-125	C	T	S	4912	8843
979	99-18637-281	C	T	A	4913	8844
980	99-18638-164	C	T	A	4914	8845
981	99-18640-458	C	T	A	4915	8846
982	99-18648-71	C	T	A	4916	8847
983	99-18666-483	G	T	A	4917	8848
984	99-18667-392	G	C	S	4918	8849
985	99-18669-223	G	C	S	4919	8850
986	99-18715-172	A	G	A	4920	8851
987	99-18719-225	C	T	S	4921	8852
988	99-18720-235	C	T	S	4922	8853
989	99-18721-442	A	G	A	4923	8854
990	99-18724-409	C	T	S	4924	8855
991	99-18729-377	A	T	S	4925	8856
992	99-1873-193	C	T	A	4926	8857
993	99-18744-170	A	G	A	4927	8858
994	99-18745-423	A	G	A	4928	8859
995	99-18747-72	C	T	S	4929	8860
996	99-18751-217	G	T	A	4930	8861
997	99-18755-267	C	T	S	4931	8862
998	99-18774-69	G	T	A	4932	8863
999	99-18775-161	G	T	A	4933	8864
1000	99-18777-130	C	T	S	4934	8865
1001	99-18802-308	G	C	S	4935	8866

1002	99-18808-155	C	T	S	4936	8867
1003	99-18814-275	G	C	S	4937	8868
1004	99-1882-289	C	T	A	4938	8869
1005	99-18822-368	C	T	S	4939	8870
1006	99-18826-378	C	T	S	4940	8871
1007	99-18827-92	A	G	A	4941	8872
1008	99-1883-121	G	T	A	4942	8873
1009	99-18847-263	C	T	S	4943	8874
1010	99-18853-64	G	T	A	4944	8875
1011	99-18855-173	A	G	A	4945	8876
1012	99-18860-308	C	T	S	4946	8877
1013	99-18861-23	C	T	S	4947	8878
1014	99-1888-162	C	T	S	4948	8879
1015	99-1890-125	C	T	A	4949	8880
1016	99-1895-67	A	C	S	4950	8881
1017	99-18974-99	A	G	A	4951	8882
1018	99-18976-135	A	T	S	4952	8883
1019	99-18982-345	C	T	S	4953	8884
1020	99-18986-248	G	C	S	4954	8885
1021	99-18987-191	A	G	A	4955	8886
1022	99-18995-300	C	T	S	4956	8887
1023	99-18996-388	A	G	A	4957	8888
1024	99-19008-237	C	T	S	4958	8889
1025	99-19013-384	C	T	S	4959	8890
1026	99-19016-51	A	G	A	4960	8891
1027	99-1909-387	G	T	A	4961	8892
1028	99-1910-94	C	T	S	4962	8893
1029	99-1916-91	G	T	A	4963	8894
1030	99-1917-434	A	T	S	4964	8895
1031	99-19253-102	A	G	A	4965	8896
1032	99-19256-149	C	T	S	4966	8897
1033	99-1934-272	A	G	A	4967	8898
1034	99-1936-289	C	T	S	4968	8899
1035	99-1944-379	C	T	S	4969	8900
1036	99-1947-205	A	G	S	4970	8901
1037	99-1948-49	G	C	S	4971	8902
1038	99-1953-287	A	G	A	4972	8903
1039	99-1955-443	A	G	A	4973	8904
1040	99-1960-424	A	T	S	4974	8905
1041	99-1964-53	C	T	S	4975	8906
1042	99-1977-440	A	G	S	4976	8907
1043	99-1997-139	G	T	A	4977	8908
1044	99-19999-92	C	T	S	4978	8909
1045	99-2000-240	G	T	A	4979	8910
1046	99-20000-252	A	G	A	4980	8911
1047	99-2001-177	A	G	S	4981	8912
1048	99-20011-229	C	T	S	4982	8913
1049	99-20018-244	G	C	S	4983	8914
1050	99-20023-386	A	T	S	4984	8915
1051	99-2003-509	G	C	S	4985	8916
1052	99-20032-90	G	T	A	4986	8917
1053	99-20033-186	G	T	A	4987	8918
1054	99-20035-283	A	C	S	4988	8919
1055	99-2004-35	C	T	S	4989	8920
1056	99-2005-466	G	C	S	4990	8921
1057	99-20057-166	C	T	S	4991	8922

1058	99-20061-56	C	T	S	4992	8923
1059	99-20062-181	A	G	A	4993	8924
1060	99-2007-278	C	T	S	4994	8925
1061	99-20074-154	A	C	S	4995	8926
1062	99-20090-81	A	C	S	4996	8927
1063	99-2010-363	C	T	A	4997	8928
1064	99-20110-65	G	T	A	4998	8929
1065	99-2012-243	A	G	A	4999	8930
1066	99-20154-451	A	T	S	5000	8931
1067	99-20156-212	A	G	A	5001	8932
1068	99-20198-54	C	T	S	5002	8933
1069	99-2020-281	C	T	A	5003	8934
1070	99-20208-176	A	G	A	5004	8935
1071	99-2022-200	A	C	S	5005	8936
1072	99-2024-132	A	G	A	5006	8937
1073	99-2025-234	C	T	A	5007	8938
1074	99-20250-362	A	T	S	5008	8939
1075	99-2027-296	A	G	S	5009	8940
1076	99-20294-274	C	T	S	5010	8941
1077	99-20303-127	C	T	S	5011	8942
1078	99-20313-311	A	G	A	5012	8943
1079	99-20320-321	C	T	S	5013	8944
1080	99-20326-130	A	G	A	5014	8945
1081	99-20332-432	A	G	A	5015	8946
1082	99-20335-48	C	T	S	5016	8947
1083	99-20340-161	A	G	A	5017	8948
1084	99-20348-403	A	G	A	5018	8949
1085	99-2035-323	C	T	S	5019	8950
1086	99-20353-229	A	G	A	5020	8951
1087	99-20357-359	A	T	S	5021	8952
1088	99-2036-168	A	T	S	5022	8953
1089	99-2037-470	C	T	S	5023	8954
1090	99-20385-215	C	T	S	5024	8955
1091	99-2041-141	A	G	A	5025	8956
1092	99-2042-439	G	C	S	5026	8957
1093	99-20420-274	C	T	S	5027	8958
1094	99-20423-430	C	T	S	5028	8959
1095	99-20424-330	C	T	S	5029	8960
1096	99-20428-271	C	T	S	5030	8961
1097	99-2043-220	A	T	S	5031	8962
1098	99-2046-275	A	G	A	5032	8963
1099	99-20469-213	C	T	S	5033	8964
1100	99-2048-267	G	C	S	5034	8965
1101	99-20480-233	C	T	S	5035	8966
1102	99-20481-131	G	C	S	5036	8967
1103	99-20485-269	A	G	A	5037	8968
1104	99-20493-238	G	T	A	5038	8969
1105	99-20499-364	A	T	S	5039	8970
1106	99-20504-90	A	G	A	5040	8971
1107	99-20508-456	C	T	S	5041	8972
1108	99-2051-360	A	C	S	5042	8973
1109	99-20511-221	C	T	S	5043	8974
1110	99-20514-71	A	G	A	5044	8975
1111	99-20518-456	A	G	A	5045	8976
1112	99-2052-376	G	T	A	5046	8977
1113	99-20527-220	A	T	S	5047	8978

1114	99-2053-386	A	G	A	5048	8979
1115	99-20531-285	A	C	S	5049	8980
1116	99-2054-93	A	G	S	5050	8981
1117	99-20542-248	A	G	A	5051	8982
1118	99-20549-141	A	G	A	5052	8983
1119	99-2055-236	A	G	A	5053	8984
1120	99-20552-37	C	T	S	5054	8985
1121	99-2056-474	C	T	S	5055	8986
1122	99-20561-126	G	T	A	5056	8987
1123	99-20565-190	C	T	S	5057	8988
1124	99-20566-376	A	G	A	5058	8989
1125	99-20567-268	C	T	S	5059	8990
1126	99-20568-284	A	C	S	5060	8991
1127	99-2058-168	G	T	A	5061	8992
1128	99-20581-125	A	T	S	5062	8993
1129	99-20594-103	G	C	S	5063	8994
1130	99-2060-322	A	T	S	5064	8995
1131	99-2061-257	A	C	S	5065	8996
1132	99-20616-287	C	T	S	5066	8997
1133	99-20623-354	C	T	S	5067	8998
1134	99-2063-451	A	G	A	5068	8999
1135	99-20639-257	C	T	S	5069	9000
1136	99-20642-382	A	G	A	5070	9001
1137	99-20651-108	A	G	A	5071	9002
1138	99-20656-171	C	T	S	5072	9003
1139	99-20659-289	C	T	S	5073	9004
1140	99-20675-407	G	C	S	5074	9005
1141	99-20677-289	C	T	S	5075	9006
1142	99-20683-98	A	C	S	5076	9007
1143	99-20688-310	A	G	A	5077	9008
1144	99-20723-206	C	T	S	5078	9009
1145	99-20726-494	A	G	A	5079	9010
1146	99-20732-413	G	C	S	5080	9011
1147	99-20738-89	G	C	S	5081	9012
1148	99-20739-335	A	G	A	5082	9013
1149	99-2074-273	A	C	S	5083	9014
1150	99-20746-369	A	G	A	5084	9015
1151	99-20747-322	A	G	A	5085	9016
1152	99-20766-117	A	G	A	5086	9017
1153	99-20768-469	C	T	S	5087	9018
1154	99-2077-510	G	C	S	5088	9019
1155	99-20771-171	A	G	A	5089	9020
1156	99-2078-348	A	G	A	5090	9021
1157	99-20797-262	C	T	S	5091	9022
1158	99-20798-87	C	T	S	5092	9023
1159	99-2080-33	A	G	A	5093	9024
1160	99-20802-358	C	T	S	5094	9025
1161	99-20814-222	C	T	S	5095	9026
1162	99-2082-284	A	G	A	5096	9027
1163	99-20823-49	C	T	S	5097	9028
1164	99-20828-131	C	T	S	5098	9029
1165	99-20830-449	A	G	A	5099	9030
1166	99-2084-504	A	G	A	5100	9031
1167	99-2085-172	C	T	S	5101	9032
1168	99-20850-374	C	T	S	5102	9033
1169	99-20853-29	A	G	A	5103	9034

1170	99-20856-158	C	T	S	5104	9035
1171	99-20867-393	A	G	A	5105	9036
1172	99-20872-325	A	C	S	5106	9037
1173	99-20883-234	C	T	S	5107	9038
1174	99-20887-420	C	T	S	5108	9039
1175	99-2089-84	A	G	A	5109	9040
1176	99-20895-36	A	G	S	5110	9041
1177	99-2092-323	A	C	S	5111	9042
1178	99-20928-66	G	C	S	5112	9043
1179	99-2093-278	C	T	S	5113	9044
1180	99-20938-256	G	T	A	5114	9045
1181	99-2094-129	A	G	A	5115	9046
1182	99-20950-251	G	C	S	5116	9047
1183	99-2098-102	G	T	A	5117	9048
1184	99-21012-277	A	C	S	5118	9049
1185	99-21021-273	C	T	S	5119	9050
1186	99-2103-270	G	C	S	5120	9051
1187	99-21035-279	C	T	S	5121	9052
1188	99-21064-278	C	T	S	5122	9053
1189	99-21070-272	A	G	A	5123	9054
1190	99-21079-169	G	T	A	5124	9055
1191	99-21084-496	C	T	S	5125	9056
1192	99-2109-276	G	T	A	5126	9057
1193	99-211-291	A	G	S	5127	9058
1194	99-21141-314	A	G	A	5128	9059
1195	99-21148-269	A	G	A	5129	9060
1196	99-21149-129	A	G	A	5130	9061
1197	99-21167-159	C	T	S	5131	9062
1198	99-2117-107	C	T	S	5132	9063
1199	99-21221-96	A	T	S	5133	9064
1200	99-2126-79	A	T	S	5134	9065
1201	99-21370-87	C	T	S	5135	9066
1202	99-2170-188	G	C	S	5136	9067
1203	99-2172-314	A	G	S	5137	9068
1204	99-2173-289	C	T	S	5138	9069
1205	99-2179-303	G	T	A	5139	9070
1206	99-2193-225	A	G	A	5140	9071
1207	99-22011-342	C	T	S	5141	9072
1208	99-22015-219	A	G	A	5142	9073
1209	99-22022-145	A	G	A	5143	9074
1210	99-22027-410	G	C	S	5144	9075
1211	99-22036-314	A	T	S	5145	9076
1212	99-22038-381	G	C	S	5146	9077
1213	99-22044-431	A	G	A	5147	9078
1214	99-22048-259	A	G	A	5148	9079
1215	99-22051-261	C	T	S	5149	9080
1216	99-22066-139	A	G	A	5150	9081
1217	99-22072-80	C	T	S	5151	9082
1218	99-22073-381	G	C	S	5152	9083
1219	99-22078-350	A	G	A	5153	9084
1220	99-22087-150	C	T	S	5154	9085
1221	99-2209-111	A	G	A	5155	9086
1222	99-22091-289	G	T	A	5156	9087
1223	99-22096-276	C	T	S	5157	9088
1224	99-22100-265	C	T	S	5158	9089
1225	99-22102-238	C	T	S	5159	9090

1226	99-22122-54	A	G	A	5160	9091
1227	99-22125-126	C	T	S	5161	9092
1228	99-2214-148	A	C	S	5162	9093
1229	99-22147-359	C	T	S	5163	9094
1230	99-22160-331	A	G	A	5164	9095
1231	99-22167-79	C	T	S	5165	9096
1232	99-22172-304	A	T	S	5166	9097
1233	99-2218-219	A	G	A	5167	9098
1234	99-22189-248	C	T	S	5168	9099
1235	99-2219-245	C	T	S	5169	9100
1236	99-22191-339	C	T	S	5170	9101
1237	99-22192-383	C	T	S	5171	9102
1238	99-222-109	C	T	S	5172	9103
1239	99-2220-300	A	G	A	5173	9104
1240	99-22209-304	A	C	S	5174	9105
1241	99-22215-391	A	G	A	5175	9106
1242	99-22217-423	G	C	S	5176	9107
1243	99-2222-459	C	T	S	5177	9108
1244	99-22227-275	A	T	S	5178	9109
1245	99-22255-384	A	G	A	5179	9110
1246	99-22262-331	C	T	S	5180	9111
1247	99-22265-294	A	C	S	5181	9112
1248	99-22266-474	C	T	S	5182	9113
1249	99-2228-301	A	G	A	5183	9114
1250	99-2229-240	G	T	A	5184	9115
1251	99-22333-237	C	T	S	5185	9116
1252	99-22336-316	C	T	S	5186	9117
1253	99-22337-199	A	C	S	5187	9118
1254	99-2235-499	G	C	S	5188	9119
1255	99-22356-370	A	C	S	5189	9120
1256	99-22357-186	C	T	S	5190	9121
1257	99-2240-281	C	T	S	5191	9122
1258	99-22409-141	C	T	S	5192	9123
1259	99-2242-206	C	T	S	5193	9124
1260	99-2244-83	A	G	A	5194	9125
1261	99-22442-147	G	T	A	5195	9126
1262	99-22449-216	G	C	S	5196	9127
1263	99-22453-370	A	T	S	5197	9128
1264	99-22456-55	A	C	S	5198	9129
1265	99-2246-340	A	G	A	5199	9130
1266	99-2248-76	C	T	S	5200	9131
1267	99-22490-246	A	G	A	5201	9132
1268	99-22491-79	G	T	A	5202	9133
1269	99-2250-236	C	T	S	5203	9134
1270	99-22503-146	C	T	S	5204	9135
1271	99-22506-395	C	T	S	5205	9136
1272	99-22513-90	A	G	A	5206	9137
1273	99-22520-413	G	C	S	5207	9138
1274	99-22546-125	C	T	S	5208	9139
1275	99-22565-114	A	G	A	5209	9140
1276	99-22571-136	C	T	S	5210	9141
1277	99-22573-321	A	G	A	5211	9142
1278	99-22578-78	C	T	S	5212	9143
1279	99-22580-72	A	T	S	5213	9144
1280	99-22585-462	G	C	S	5214	9145
1281	99-22586-39	A	G	S	5215	9146

1282	99-22604-208	G	T	A	5216	9147
1283	99-22610-343	A	G	A	5217	9148
1284	99-22615-392	C	T	S	5218	9149
1285	99-22617-378	C	T	S	5219	9150
1286	99-22620-404	C	T	S	5220	9151
1287	99-22628-292	A	G	A	5221	9152
1288	99-22629-124	C	T	S	5222	9153
1289	99-22632-237	G	C	S	5223	9154
1290	99-22646-233	A	G	A	5224	9155
1291	99-22648-57	C	T	S	5225	9156
1292	99-22650-64	A	C	S	5226	9157
1293	99-22652-343	A	G	A	5227	9158
1294	99-22655-319	A	T	S	5228	9159
1295	99-22660-386	A	G	A	5229	9160
1296	99-22662-268	A	G	A	5230	9161
1297	99-22666-164	C	T	S	5231	9162
1298	99-22668-232	G	T	A	5232	9163
1299	99-22674-31	C	T	S	5233	9164
1300	99-22675-187	A	G	A	5234	9165
1301	99-22680-130	C	T	S	5235	9166
1302	99-22683-107	A	G	A	5236	9167
1303	99-2269-179	A	G	A	5237	9168
1304	99-22700-358	A	G	A	5238	9169
1305	99-22701-307	C	T	S	5239	9170
1306	99-2271-403	A	G	A	5240	9171
1307	99-22712-242	A	G	A	5241	9172
1308	99-22718-94	A	T	S	5242	9173
1309	99-2272-409	G	T	A	5243	9174
1310	99-22728-207	A	G	A	5244	9175
1311	99-2273-528	C	T	S	5245	9176
1312	99-22733-281	G	C	S	5246	9177
1313	99-22741-180	A	G	A	5247	9178
1314	99-2275-466	C	T	S	5248	9179
1315	99-2276-331	C	T	S	5249	9180
1316	99-22771-150	A	G	A	5250	9181
1317	99-22775-365	C	T	S	5251	9182
1318	99-2278-276	A	G	A	5252	9183
1319	99-22785-431	A	T	S	5253	9184
1320	99-22843-342	G	T	A	5254	9185
1321	99-22844-211	A	G	A	5255	9186
1322	99-22857-88	C	T	S	5256	9187
1323	99-22868-425	A	C	S	5257	9188
1324	99-22872-431	C	T	S	5258	9189
1325	99-2288-144	C	T	S	5259	9190
1326	99-22917-145	G	T	A	5260	9191
1327	99-22937-395	C	T	S	5261	9192
1328	99-22948-262	C	T	S	5262	9193
1329	99-22954-306	A	C	S	5263	9194
1330	99-22957-409	A	G	A	5264	9195
1331	99-22959-239	A	G	A	5265	9196
1332	99-22964-82	C	T	S	5266	9197
1333	99-22975-126	C	T	S	5267	9198
1334	99-23014-300	A	G	A	5268	9199
1335	99-23018-166	A	G	A	5269	9200
1336	99-23020-187	G	T	A	5270	9201
1337	99-23083-59	C	T	S	5271	9202

1338	99-23100-367	A	G	A	5272	9203
1339	99-23115-404	G	C	S	5273	9204
1340	99-23118-402	A	G	A	5274	9205
1341	99-2312-358	C	T	S	5275	9206
1342	99-23123-250	A	G	A	5276	9207
1343	99-23127-314	G	C	S	5277	9208
1344	99-23132-192	A	G	A	5278	9209
1345	99-23134-89	A	G	A	5279	9210
1346	99-2315-213	A	G	A	5280	9211
1347	99-23150-262	A	C	S	5281	9212
1348	99-2320-292	C	T	S	5282	9213
1349	99-23201-345	C	T	S	5283	9214
1350	99-23202-185	A	C	S	5284	9215
1351	99-23204-262	C	T	S	5285	9216
1352	99-23207-281	C	T	S	5286	9217
1353	99-2321-82	C	T	S	5287	9218
1354	99-23228-176	G	C	S	5288	9219
1355	99-2324-338	A	C	S	5289	9220
1356	99-23266-146	A	G	A	5290	9221
1357	99-23269-263	A	T	S	5291	9222
1358	99-2328-535	G	C	S	5292	9223
1359	99-23299-424	A	G	A	5293	9224
1360	99-23302-326	C	T	S	5294	9225
1361	99-2331-639	G	T	A	5295	9226
1362	99-23312-93	A	G	A	5296	9227
1363	99-23317-51	A	G	A	5297	9228
1364	99-23322-49	A	G	A	5298	9229
1365	99-23326-120	A	G	A	5299	9230
1366	99-23328-292	A	G	A	5300	9231
1367	99-23333-157	A	G	A	5301	9232
1368	99-23334-443	A	G	A	5302	9233
1369	99-23359-99	G	C	S	5303	9234
1370	99-23381-412	A	G	A	5304	9235
1371	99-23387-404	G	C	S	5305	9236
1372	99-23413-242	A	G	A	5306	9237
1373	99-23415-131	A	G	A	5307	9238
1374	99-23417-128	G	T	A	5308	9239
1375	99-23437-347	A	G	A	5309	9240
1376	99-23440-274	A	G	A	5310	9241
1377	99-23444-203	A	G	A	5311	9242
1378	99-2345-28	G	C	A	5312	9243
1379	99-23451-78	A	G	A	5313	9244
1380	99-23452-306	G	T	A	5314	9245
1381	99-23454-317	C	T	A	5315	9246
1382	99-23460-199	A	C	S	5316	9247
1383	99-23462-192	C	T	S	5317	9248
1384	99-23463-118	C	T	S	5318	9249
1385	99-23469-288	C	T	S	5319	9250
1386	99-2347-207	A	C	S	5320	9251
1387	99-23473-35	C	T	S	5321	9252
1388	99-2348-127	A	G	A	5322	9253
1389	99-23488-239	A	G	A	5323	9254
1390	99-23492-151	C	T	S	5324	9255
1391	99-23496-94	A	G	A	5325	9256
1392	99-23510-45	A	G	A	5326	9257
1393	99-23528-452	C	T	S	5327	9258

1394	99-2356-322	A	G	A	5328	9259
1395	99-2362-270	A	G	A	5329	9260
1396	99-2364-329	G	C	S	5330	9261
1397	99-2367-61	A	G	A	5331	9262
1398	99-2368-61	A	G	A	5332	9263
1399	99-23687-107	C	T	A	5333	9264
1400	99-237-151	A	G	A	5334	9265
1401	99-23714-196	G	C	S	5335	9266
1402	99-23737-186	C	T	S	5336	9267
1403	99-2375-114	C	T	A	5337	9268
1404	99-23773-199	C	T	S	5338	9269
1405	99-2378-200	A	G	A	5339	9270
1406	99-2381-394	A	G	A	5340	9271
1407	99-2409-298	A	G	A	5341	9272
1408	99-241-341	A	T	S	5342	9273
1409	99-2413-368	A	G	A	5343	9274
1410	99-2417-177	C	T	S	5344	9275
1411	99-2419-285	C	T	S	5345	9276
1412	99-24246-247	C	T	A	5346	9277
1413	99-24253-437	A	G	S	5347	9278
1414	99-24259-466	A	T	S	5348	9279
1415	99-24264-380	G	C	S	5349	9280
1416	99-24269-417	C	T	A	5350	9281
1417	99-24270-207	G	T	A	5351	9282
1418	99-24275-107	A	G	S	5352	9283
1419	99-24284-213	A	T	S	5353	9284
1420	99-24286-231	A	G	S	5354	9285
1421	99-24288-121	A	G	S	5355	9286
1422	99-24333-37	A	G	S	5356	9287
1423	99-24342-311	C	T	A	5357	9288
1424	99-24376-24	A	G	S	5358	9289
1425	99-24379-319	C	T	S	5359	9290
1426	99-24381-217	A	G	S	5360	9291
1427	99-24385-210	A	T	S	5361	9292
1428	99-24388-391	A	T	S	5362	9293
1429	99-24390-27	A	G	S	5363	9294
1430	99-24392-61	A	C	S	5364	9295
1431	99-24393-108	A	G	S	5365	9296
1432	99-2440-246	C	T	S	5366	9297
1433	99-24409-383	A	G	S	5367	9298
1434	99-24411-420	G	C	S	5368	9299
1435	99-24427-321	A	G	S	5369	9300
1436	99-24432-284	A	C	S	5370	9301
1437	99-24438-402	A	G	S	5371	9302
1438	99-24441-431	C	T	A	5372	9303
1439	99-24447-448	A	T	S	5373	9304
1440	99-2445-79	C	T	S	5374	9305
1441	99-24454-257	G	C	S	5375	9306
1442	99-24463-206	A	G	S	5376	9307
1443	99-24496-171	C	T	A	5377	9308
1444	99-24506-396	A	G	S	5378	9309
1445	99-24508-45	G	C	S	5379	9310
1446	99-24529-330	A	G	S	5380	9311
1447	99-24534-317	G	C	S	5381	9312
1448	99-24554-324	A	G	A	5382	9313
1449	99-24557-406	G	T	A	5383	9314

1450	99-24561-360	A	T	S	5384	9315
1451	99-24570-260	G	C	S	5385	9316
1452	99-24688-312	C	T	A	5386	9317
1453	99-24725-138	A	G	S	5387	9318
1454	99-24727-360	A	G	S	5388	9319
1455	99-24750-293	C	T	A	5389	9320
1456	99-24778-221	C	T	A	5390	9321
1457	99-24793-390	C	T	A	5391	9322
1458	99-24800-565	G	T	S	5392	9323
1459	99-25005-154	A	C	S	5393	9324
1460	99-25007-131	A	T	S	5394	9325
1461	99-25053-114	A	G	S	5395	9326
1462	99-25055-44	A	G	S	5396	9327
1463	99-25070-78	C	T	A	5397	9328
1464	99-25077-124	A	G	S	5398	9329
1465	99-25129-166	C	T	A	5399	9330
1466	99-25134-296	A	G	S	5400	9331
1467	99-2524-98	A	G	A	5401	9332
1468	99-25246-170	C	T	A	5402	9333
1469	99-25249-151	G	T	A	5403	9334
1470	99-2525-142	A	G	S	5404	9335
1471	99-25255-288	C	T	A	5405	9336
1472	99-25369-121	C	T	S	5406	9337
1473	99-25379-389	C	T	S	5407	9338
1474	99-25382-226	A	C	S	5408	9339
1475	99-25387-220	G	T	A	5409	9340
1476	99-25400-379	C	T	S	5410	9341
1477	99-25412-354	G	T	A	5411	9342
1478	99-25431-269	A	C	S	5412	9343
1479	99-25432-119	C	T	S	5413	9344
1480	99-25433-351	A	G	S	5414	9345
1481	99-25447-272	A	G	S	5415	9346
1482	99-25448-348	G	T	A	5416	9347
1483	99-25452-83	C	T	A	5417	9348
1484	99-25454-349	G	T	A	5418	9349
1485	99-25458-103	G	C	S	5419	9350
1486	99-25503-333	A	T	S	5420	9351
1487	99-25507-373	C	T	A	5421	9352
1488	99-25510-390	A	C	S	5422	9353
1489	99-25538-423	A	C	S	5423	9354
1490	99-25539-86	C	T	A	5424	9355
1491	99-25543-390	A	G	S	5425	9356
1492	99-25575-303	C	T	A	5426	9357
1493	99-25618-196	A	C	S	5427	9358
1494	99-25620-360	C	T	A	5428	9359
1495	99-25629-262	A	G	S	5429	9360
1496	99-25657-314	C	T	A	5430	9361
1497	99-25672-97	A	G	S	5431	9362
1498	99-25676-211	C	T	A	5432	9363
1499	99-25678-307	A	G	S	5433	9364
1500	99-2570-218	C	T	S	5434	9365
1501	99-25712-418	C	T	A	5435	9366
1502	99-25716-393	C	T	A	5436	9367
1503	99-25717-252	G	T	A	5437	9368
1504	99-25725-80	A	G	S	5438	9369
1505	99-25732-152	A	G	S	5439	9370

1506	99-25745-36	A	G	S	5440	9371
1507	99-25781-275	C	T	A	5441	9372
1508	99-25836-106	C	T	S	5442	9373
1509	99-2597-34	C	T	S	5443	9374
1510	99-26001-224	A	G	S	5444	9375
1511	99-26002-93	C	T	A	5445	9376
1512	99-26042-310	A	G	A	5446	9377
1513	99-26080-152	C	T	S	5447	9378
1514	99-26082-48	C	T	S	5448	9379
1515	99-26099-119	A	G	A	5449	9380
1516	99-2610-121	A	C	S	5450	9381
1517	99-26105-273	A	C	S	5451	9382
1518	99-26116-191	C	T	A	5452	9383
1519	99-2615-83	C	T	S	5453	9384
1520	99-2620-227	A	G	A	5454	9385
1521	99-2624-407	G	T	A	5455	9386
1522	99-2625-70	A	G	A	5456	9387
1523	99-2637-28	A	G	A	5457	9388
1524	99-2662-407	C	T	S	5458	9389
1525	99-2669-233	A	G	A	5459	9390
1526	99-2675-121	A	G	A	5460	9391
1527	99-2683-388	C	T	S	5461	9392
1528	99-342-288	A	C	S	5462	9393
1529	99-370-205	A	G	A	5463	9394
1530	99-371-415	C	T	S	5464	9395
1531	99-388-405	A	G	A	5465	9396
1532	99-390-246	G	T	S	5466	9397
1533	99-393-448	A	T	S	5467	9398
1534	99-397-205	A	G	A	5468	9399
1535	99-400-102	G	C	S	5469	9400
1536	99-402-139	A	G	A	5470	9401
1537	99-404-114	C	T	S	5471	9402
1538	99-414-349	G	C	S	5472	9403
1539	99-417-241	A	G	A	5473	9404
1540	99-426-359	G	T	S	5474	9405
1541	99-429-115	A	C	S	5475	9406
1542	99-430-352	C	T	S	5476	9407
1543	99-435-41	A	G	A	5477	9408
1544	99-449-344	G	T	S	5478	9409
1545	99-4536-255	A	G	A	5479	9410
1546	99-4541-39	G	T	A	5480	9411
1547	99-4544-287	A	G	A	5481	9412
1548	99-4547-312	C	T	S	5482	9413
1549	99-4595-341	A	T	S	5483	9414
1550	99-4604-26	A	T	S	5484	9415
1551	99-4618-240	C	T	S	5485	9416
1552	99-4625-216	C	T	S	5486	9417
1553	99-4630-272	A	G	A	5487	9418
1554	99-4644-107	A	T	S	5488	9419
1555	99-465-443	A	C	S	5489	9420
1556	99-4655-145	C	T	S	5490	9421
1557	99-466-361	C	T	S	5491	9422
1558	99-4666-185	C	T	S	5492	9423
1559	99-4674-166	A	G	A	5493	9424
1560	99-4676-342	A	G	S	5494	9425
1561	99-4677-58	C	T	S	5495	9426

1562	99-4679-240	C	T	A	5496	9427
1563	99-4680-352	C	T	S	5497	9428
1564	99-4681-228	A	G	A	5498	9429
1565	99-4682-177	C	T	S	5499	9430
1566	99-4685-217	A	G	A	5500	9431
1567	99-4705-226	G	C	S	5501	9432
1568	99-4714-156	A	G	A	5502	9433
1569	99-472-70	C	T	S	5503	9434
1570	99-4725-251	A	T	S	5504	9435
1571	99-4756-236	C	T	S	5505	9436
1572	99-4761-279	A	G	A	5506	9437
1573	99-477-302	A	G	A	5507	9438
1574	99-4777-137	G	C	S	5508	9439
1575	99-4790-305	A	G	A	5509	9440
1576	99-4796-325	A	C	S	5510	9441
1577	99-482-130	C	T	S	5511	9442
1578	99-4822-291	A	G	A	5512	9443
1579	99-4823-173	G	T	A	5513	9444
1580	99-483-424	A	C	S	5514	9445
1581	99-4836-206	A	G	A	5515	9446
1582	99-4838-424	C	T	S	5516	9447
1583	99-4840-368	C	T	S	5517	9448
1584	99-4844-102	C	T	S	5518	9449
1585	99-486-243	C	T	S	5519	9450
1586	99-4863-240	C	T	S	5520	9451
1587	99-4882-351	A	T	S	5521	9452
1588	99-4890-255	G	T	A	5522	9453
1589	99-4891-509	A	C	S	5523	9454
1590	99-4895-158	C	T	S	5524	9455
1591	99-490-202	C	T	S	5525	9456
1592	99-4924-254	C	T	S	5526	9457
1593	99-4928-102	C	T	S	5527	9458
1594	99-4950-196	A	C	S	5528	9459
1595	99-4951-36	C	T	A	5529	9460
1596	99-4956-236	A	G	A	5530	9461
1597	99-4966-298	A	G	A	5531	9462
1598	99-4968-273	C	T	S	5532	9463
1599	99-5016-206	C	T	S	5533	9464
1600	99-5029-240	G	T	A	5534	9465
1601	99-5032-232	A	C	S	5535	9466
1602	99-5036-40	C	T	S	5536	9467
1603	99-5038-181	C	T	S	5537	9468
1604	99-5043-111	A	C	S	5538	9469
1605	99-5099-245	A	G	A	5539	9470
1606	99-5101-284	G	T	A	5540	9471
1607	99-5104-160	A	G	A	5541	9472
1608	99-5107-184	G	T	A	5542	9473
1609	99-5108-144	A	G	A	5543	9474
1610	99-511-33	C	T	S	5544	9475
1611	99-5130-355	C	T	S	5545	9476
1612	99-5142-74	C	T	S	5546	9477
1613	99-5148-269	G	C	S	5547	9478
1614	99-5149-436	C	T	S	5548	9479
1615	99-5157-422	G	C	S	5549	9480
1616	99-5162-461	C	T	S	5550	9481
1617	99-5168-220	C	T	S	5551	9482

1618	99-5184-146	A	G	A	5552	9483
1619	99-5186-455	A	G	A	5553	9484
1620	99-5189-412	C	T	S	5554	9485
1621	99-5193-430	A	G	A	5555	9486
1622	99-5194-145	A	G	A	5556	9487
1623	99-5199-108	A	T	S	5557	9488
1624	99-5202-145	A	G	A	5558	9489
1625	99-5224-293	G	C	S	5559	9490
1626	99-5225-198	C	T	S	5560	9491
1627	99-5226-215	A	G	A	5561	9492
1628	99-5247-158	A	G	A	5562	9493
1629	99-5252-252	A	G	A	5563	9494
1630	99-5265-288	C	T	S	5564	9495
1631	99-5290-322	C	T	S	5565	9496
1632	99-5291-331	A	C	S	5566	9497
1633	99-5294-362	A	C	S	5567	9498
1634	99-5306-93	C	T	S	5568	9499
1635	99-5308-341	G	C	S	5569	9500
1636	99-5312-273	C	T	S	5570	9501
1637	99-5326-332	A	G	A	5571	9502
1638	99-5338-151	C	T	S	5572	9503
1639	99-5355-165	A	G	A	5573	9504
1640	99-5356-100	A	T	S	5574	9505
1641	99-5360-151	C	T	S	5575	9506
1642	99-5362-203	C	T	S	5576	9507
1643	99-5364-95	C	T	A	5577	9508
1644	99-5379-158	A	G	A	5578	9509
1645	99-5386-85	A	G	A	5579	9510
1646	99-5389-409	A	G	A	5580	9511
1647	99-5390-375	C	T	S	5581	9512
1648	99-5401-280	C	T	S	5582	9513
1649	99-5405-376	C	T	S	5583	9514
1650	99-5406-299	A	T	S	5584	9515
1651	99-5407-173	C	T	S	5585	9516
1652	99-5411-378	C	T	S	5586	9517
1653	99-5416-137	A	G	A	5587	9518
1654	99-5420-425	G	C	S	5588	9519
1655	99-5427-466	A	G	A	5589	9520
1656	99-5432-391	G	C	S	5590	9521
1657	99-5433-45	C	T	S	5591	9522
1658	99-5437-159	A	G	A	5592	9523
1659	99-5438-70	C	T	S	5593	9524
1660	99-5441-287	C	T	S	5594	9525
1661	99-5446-303	C	T	S	5595	9526
1662	99-5447-322	A	G	A	5596	9527
1663	99-5458-203	C	T	S	5597	9528
1664	99-5468-319	A	C	S	5598	9529
1665	99-5472-290	C	T	S	5599	9530
1666	99-5475-455	A	G	A	5600	9531
1667	99-5477-207	A	C	S	5601	9532
1668	99-5485-325	A	G	A	5602	9533
1669	99-5490-368	C	T	S	5603	9534
1670	99-5494-205	A	G	A	5604	9535
1671	99-5502-433	A	C	S	5605	9536
1672	99-5505-226	C	T	S	5606	9537
1673	99-5516-121	C	T	S	5607	9538

1674	99-5526-334	C	T	S	5608	9539
1675	99-5566-131	A	G	A	5609	9540
1676	99-5582-71	G	C	S	5610	9541
1677	99-5590-99	C	T	S	5611	9542
1678	99-5595-380	A	G	A	5612	9543
1679	99-5596-216	A	G	A	5613	9544
1680	99-5604-376	A	G	A	5614	9545
1681	99-5608-324	A	G	A	5615	9546
1682	99-5632-425	A	G	A	5616	9547
1683	99-5633-334	A	G	A	5617	9548
1684	99-5634-426	C	T	S	5618	9549
1685	99-5636-198	C	T	S	5619	9550
1686	99-5660-265	G	C	S	5620	9551
1687	99-5670-264	C	T	S	5621	9552
1688	99-5678-321	A	G	A	5622	9553
1689	99-5680-109	A	G	A	5623	9554
1690	99-5681-81	C	T	S	5624	9555
1691	99-5685-274	A	T	S	5625	9556
1692	99-5686-274	G	C	S	5626	9557
1693	99-5700-142	G	C	S	5627	9558
1694	99-5702-192	C	T	S	5628	9559
1695	99-5703-72	C	T	S	5629	9560
1696	99-5709-80	A	G	A	5630	9561
1697	99-5711-206	C	T	S	5631	9562
1698	99-5712-123	A	T	S	5632	9563
1699	99-5727-77	C	T	S	5633	9564
1700	99-5729-370	A	G	A	5634	9565
1701	99-5731-450	G	T	A	5635	9566
1702	99-5741-59	A	G	A	5636	9567
1703	99-5742-337	C	T	S	5637	9568
1704	99-5745-256	G	C	S	5638	9569
1705	99-5756-233	A	G	A	5639	9570
1706	99-576-421	G	C	S	5640	9571
1707	99-5760-164	A	G	A	5641	9572
1708	99-5770-275	C	T	S	5642	9573
1709	99-5781-110	G	C	S	5643	9574
1710	99-5795-234	A	C	S	5644	9575
1711	99-5813-34	A	T	S	5645	9576
1712	99-582-132	A	G	S	5646	9577
1713	99-5832-136	C	T	S	5647	9578
1714	99-5836-327	C	T	S	5648	9579
1715	99-5837-407	C	T	S	5649	9580
1716	99-5860-278	A	G	A	5650	9581
1717	99-5867-284	G	T	A	5651	9582
1718	99-5875-411	A	G	A	5652	9583
1719	99-5893-211	A	G	A	5653	9584
1720	99-5907-143	C	T	S	5654	9585
1721	99-5908-225	A	G	A	5655	9586
1722	99-5909-292	C	T	S	5656	9587
1723	99-5912-49	A	G	A	5657	9588
1724	99-5915-378	A	G	A	5658	9589
1725	99-5951-438	C	T	S	5659	9590
1726	99-5957-123	G	C	S	5660	9591
1727	99-596-228	G	C	S	5661	9592
1728	99-5968-382	A	T	S	5662	9593
1729	99-5979-96	C	T	S	5663	9594

1730	99-598-130	A	G	A	5664	9595
1731	99-6007-246	A	C	S	5665	9596
1732	99-6012-220	G	T	A	5666	9597
1733	99-602-258	A	G	A	5667	9598
1734	99-6038-286	A	G	S	5668	9599
1735	99-6042-134	G	C	S	5669	9600
1736	99-6051-251	G	C	S	5670	9601
1737	99-6067-247	C	T	S	5671	9602
1738	99-6069-41	A	G	A	5672	9603
1739	99-607-397	A	G	A	5673	9604
1740	99-6077-346	C	T	S	5674	9605
1741	99-6079-343	A	G	A	5675	9606
1742	99-608-183	G	T	A	5676	9607
1743	99-6080-99	C	T	S	5677	9608
1744	99-609-225	A	T	S	5678	9609
1745	99-6091-305	A	G	A	5679	9610
1746	99-6094-223	C	T	S	5680	9611
1747	99-6095-316	A	G	A	5681	9612
1748	99-6097-202	G	T	A	5682	9613
1749	99-610-250	A	G	A	5683	9614
1750	99-6112-275	C	T	S	5684	9615
1751	99-6117-221	A	G	A	5685	9616
1752	99-6122-100	A	G	A	5686	9617
1753	99-6131-166	A	G	A	5687	9618
1754	99-6135-319	C	T	S	5688	9619
1755	99-614-346	G	C	S	5689	9620
1756	99-6141-339	C	T	S	5690	9621
1757	99-615-387	A	C	S	5691	9622
1758	99-616-338	A	G	A	5692	9623
1759	99-6176-96	A	G	A	5693	9624
1760	99-6180-389	G	T	A	5694	9625
1761	99-6181-328	A	C	S	5695	9626
1762	99-6189-224	A	G	A	5696	9627
1763	99-619-141	C	T	S	5697	9628
1764	99-6191-252	A	C	S	5698	9629
1765	99-6193-88	C	T	S	5699	9630
1766	99-621-215	A	G	A	5700	9631
1767	99-6217-420	C	T	S	5701	9632
1768	99-622-95	A	G	A	5702	9633
1769	99-6253-308	C	T	S	5703	9634
1770	99-6257-226	C	T	S	5704	9635
1771	99-6261-172	A	C	S	5705	9636
1772	99-6278-391	C	T	S	5706	9637
1773	99-6294-184	C	T	S	5707	9638
1774	99-6298-280	A	C	S	5708	9639
1775	99-6300-106	G	C	S	5709	9640
1776	99-6310-217	G	C	S	5710	9641
1777	99-632-173	C	T	S	5711	9642
1778	99-6327-270	A	C	S	5712	9643
1779	99-6332-143	C	T	S	5713	9644
1780	99-6367-268	A	C	S	5714	9645
1781	99-6368-426	A	G	A	5715	9646
1782	99-6404-147	A	G	A	5716	9647
1783	99-6409-62	C	T	S	5717	9648
1784	99-6411-93	A	G	A	5718	9649
1785	99-6413-369	C	T	S	5719	9650

1786	99-6415-279	C	T	S	5720	9651
1787	99-6421-210	C	T	S	5721	9652
1788	99-6423-90	G	C	S	5722	9653
1789	99-6426-413	A	G	A	5723	9654
1790	99-6427-190	A	G	A	5724	9655
1791	99-6435-343	A	G	A	5725	9656
1792	99-6437-77	A	C	S	5726	9657
1793	99-6440-318	G	C	S	5727	9658
1794	99-6447-178	C	T	S	5728	9659
1795	99-6456-165	C	T	S	5729	9660
1796	99-6459-201	A	G	A	5730	9661
1797	99-646-271	C	T	S	5731	9662
1798	99-6463-348	A	C	S	5732	9663
1799	99-6468-288	A	G	A	5733	9664
1800	99-6478-358	A	G	A	5734	9665
1801	99-6480-440	A	G	A	5735	9666
1802	99-6489-237	G	T	A	5736	9667
1803	99-649-422	A	T	S	5737	9668
1804	99-6496-340	A	G	A	5738	9669
1805	99-6511-176	C	T	S	5739	9670
1806	99-6525-196	C	T	S	5740	9671
1807	99-6527-95	A	C	S	5741	9672
1808	99-6529-519	C	T	S	5742	9673
1809	99-6539-298	A	G	A	5743	9674
1810	99-6557-401	A	G	A	5744	9675
1811	99-658-367	A	G	A	5745	9676
1812	99-6581-45	C	T	S	5746	9677
1813	99-6586-359	A	G	A	5747	9678
1814	99-6588-94	C	T	S	5748	9679
1815	99-6595-322	G	C	S	5749	9680
1816	99-6609-103	C	T	S	5750	9681
1817	99-6612-185	G	T	A	5751	9682
1818	99-6613-223	G	C	S	5752	9683
1819	99-6620-294	A	C	S	5753	9684
1820	99-6628-474	A	C	S	5754	9685
1821	99-6639-290	C	T	S	5755	9686
1822	99-6640-342	A	T	S	5756	9687
1823	99-6646-465	A	G	A	5757	9688
1824	99-6667-63	C	T	S	5758	9689
1825	99-6672-314	G	T	A	5759	9690
1826	99-6675-324	A	G	A	5760	9691
1827	99-6688-363	A	G	A	5761	9692
1828	99-669-291	A	C	S	5762	9693
1829	99-6697-80	A	T	S	5763	9694
1830	99-670-274	A	G	A	5764	9695
1831	99-6705-101	C	T	S	5765	9696
1832	99-6706-308	C	T	S	5766	9697
1833	99-6715-439	C	T	S	5767	9698
1834	99-6726-341	A	T	S	5768	9699
1835	99-6730-356	A	G	A	5769	9700
1836	99-6753-79	A	C	S	5770	9701
1837	99-6757-288	G	T	A	5771	9702
1838	99-676-357	A	G	A	5772	9703
1839	99-6781-263	G	T	A	5773	9704
1840	99-6790-378	C	T	S	5774	9705
1841	99-680-228	A	G	A	5775	9706

1842	99-6804-426	A	G	A	5776	9707
1843	99-6815-484	G	C	S	5777	9708
1844	99-6820-251	A	G	A	5778	9709
1845	99-6832-178	C	T	S	5779	9710
1846	99-6856-433	A	G	A	5780	9711
1847	99-6865-455	C	T	S	5781	9712
1848	99-6866-130	A	G	A	5782	9713
1849	99-6869-256	A	G	A	5783	9714
1850	99-6876-229	C	T	S	5784	9715
1851	99-689-219	C	T	S	5785	9716
1852	99-6893-392	C	T	S	5786	9717
1853	99-6895-144	A	T	S	5787	9718
1854	99-6938-347	G	T	S	5788	9719
1855	99-694-236	G	T	A	5789	9720
1856	99-6940-464	A	G	A	5790	9721
1857	99-6942-313	A	C	S	5791	9722
1858	99-6951-410	G	T	A	5792	9723
1859	99-6956-58	C	T	S	5793	9724
1860	99-6957-137	G	C	S	5794	9725
1861	99-6960-412	A	T	S	5795	9726
1862	99-6962-34	G	C	S	5796	9727
1863	99-6979-64	C	T	S	5797	9728
1864	99-6986-157	A	G	A	5798	9729
1865	99-6988-236	A	C	S	5799	9730
1866	99-6989-397	A	G	A	5800	9731
1867	99-6996-217	C	T	S	5801	9732
1868	99-700-123	A	G	A	5802	9733
1869	99-7000-235	G	C	S	5803	9734
1870	99-7004-304	A	G	A	5804	9735
1871	99-7013-250	C	T	S	5805	9736
1872	99-7024-122	G	T	A	5806	9737
1873	99-7025-226	C	T	S	5807	9738
1874	99-7026-247	C	T	S	5808	9739
1875	99-7047-225	C	T	S	5809	9740
1876	99-7056-49	A	G	A	5810	9741
1877	99-708-243	C	T	S	5811	9742
1878	99-7084-187	C	T	S	5812	9743
1879	99-7090-294	A	G	A	5813	9744
1880	99-7093-36	A	C	S	5814	9745
1881	99-7098-382	A	G	A	5815	9746
1882	99-7103-155	C	T	S	5816	9747
1883	99-7104-187	A	G	A	5817	9748
1884	99-7107-143	A	G	A	5818	9749
1885	99-7114-31	A	G	A	5819	9750
1886	99-7119-278	A	G	A	5820	9751
1887	99-7129-335	A	C	S	5821	9752
1888	99-7131-259	C	T	S	5822	9753
1889	99-7136-329	C	T	S	5823	9754
1890	99-7137-420	C	T	S	5824	9755
1891	99-7140-355	A	G	A	5825	9756
1892	99-7141-395	A	G	A	5826	9757
1893	99-7144-261	C	T	S	5827	9758
1894	99-7148-262	C	T	S	5828	9759
1895	99-7167-438	A	G	A	5829	9760
1896	99-7172-441	A	G	A	5830	9761
1897	99-7177-81	C	T	S	5831	9762

1898	99-718-261	A	G	A	5832	9763
1899	99-7183-338	A	G	A	5833	9764
1900	99-7193-228	G	C	S	5834	9765
1901	99-72-109	C	T	S	5835	9766
1902	99-7212-346	C	T	S	5836	9767
1903	99-7214-109	C	T	S	5837	9768
1904	99-7218-444	C	T	S	5838	9769
1905	99-7234-101	A	T	S	5839	9770
1906	99-724-246	A	G	A	5840	9771
1907	99-7252-279	C	T	S	5841	9772
1908	99-7274-172	C	T	S	5842	9773
1909	99-7275-150	C	T	S	5843	9774
1910	99-7276-286	A	G	A	5844	9775
1911	99-7293-201	A	G	A	5845	9776
1912	99-73-140	C	T	S	5846	9777
1913	99-7311-179	A	G	A	5847	9778
1914	99-7312-177	A	G	A	5848	9779
1915	99-7323-178	A	C	S	5849	9780
1916	99-7326-94	G	C	S	5850	9781
1917	99-7334-350	C	T	S	5851	9782
1918	99-734-126	C	T	S	5852	9783
1919	99-7349-384	G	C	S	5853	9784
1920	99-7356-176	A	G	A	5854	9785
1921	99-7363-474	C	T	S	5855	9786
1922	99-737-372	A	G	A	5856	9787
1923	99-7373-339	A	G	A	5857	9788
1924	99-7374-230	A	G	A	5858	9789
1925	99-7375-210	C	T	S	5859	9790
1926	99-7376-157	A	T	S	5860	9791
1927	99-7380-255	A	G	A	5861	9792
1928	99-7387-414	C	T	S	5862	9793
1929	99-7391-356	C	T	S	5863	9794
1930	99-7396-228	A	T	S	5864	9795
1931	99-7402-110	C	T	S	5865	9796
1932	99-7405-92	C	T	S	5866	9797
1933	99-7406-380	A	G	A	5867	9798
1934	99-7417-440	C	T	S	5868	9799
1935	99-7429-204	G	T	A	5869	9800
1936	99-7447-281	G	C	S	5870	9801
1937	99-7453-405	C	T	S	5871	9802
1938	99-7454-35	G	C	S	5872	9803
1939	99-747-252	A	G	A	5873	9804
1940	99-7475-179	G	T	A	5874	9805
1941	99-7480-66	A	G	A	5875	9806
1942	99-7492-275	C	T	S	5876	9807
1943	99-7493-249	G	C	S	5877	9808
1944	99-7502-382	A	T	S	5878	9809
1945	99-7504-342	A	C	S	5879	9810
1946	99-7520-222	A	G	A	5880	9811
1947	99-7524-130	A	G	A	5881	9812
1948	99-7543-467	A	G	A	5882	9813
1949	99-755-83	A	T	S	5883	9814
1950	99-7598-388	A	G	A	5884	9815
1951	99-760-261	C	T	S	5885	9816
1952	99-7604-309	A	G	A	5886	9817
1953	99-7605-62	G	C	S	5887	9818

1954	99-7608-388	C	T	S	5888	9819
1955	99-7610-444	C	T	S	5889	9820
1956	99-7611-156	A	G	A	5890	9821
1957	99-7614-28	A	T	S	5891	9822
1958	99-763-240	A	G	A	5892	9823
1959	99-7642-191	A	G	A	5893	9824
1960	99-7643-350	C	T	S	5894	9825
1961	99-7650-187	G	C	S	5895	9826
1962	99-7671-33	G	C	S	5896	9827
1963	99-7677-107	C	T	S	5897	9828
1964	99-7688-325	C	T	S	5898	9829
1965	99-7692-340	A	G	A	5899	9830
1966	99-77-318	A	C	A	5900	9831
1967	99-7706-303	A	G	A	5901	9832
1968	99-771-391	A	G	A	5902	9833
1969	99-7710-318	C	T	S	5903	9834
1970	99-7712-176	C	T	S	5904	9835
1971	99-7721-379	A	C	S	5905	9836
1972	99-7727-65	C	T	S	5906	9837
1973	99-7728-334	A	G	A	5907	9838
1974	99-7732-122	C	T	S	5908	9839
1975	99-7737-264	A	G	A	5909	9840
1976	99-7744-255	G	C	S	5910	9841
1977	99-7745-305	G	C	S	5911	9842
1978	99-7749-123	C	T	S	5912	9843
1979	99-7751-450	A	G	A	5913	9844
1980	99-7753-199	G	C	S	5914	9845
1981	99-7754-119	G	T	A	5915	9846
1982	99-7759-63	G	T	A	5916	9847
1983	99-7762-227	A	G	A	5917	9848
1984	99-7764-161	A	G	A	5918	9849
1985	99-7775-313	C	T	A	5919	9850
1986	99-7784-31	C	T	S	5920	9851
1987	99-7789-404	G	T	A	5921	9852
1988	99-7792-173	C	T	S	5922	9853
1989	99-7796-130	G	C	S	5923	9854
1990	99-7803-253	A	G	A	5924	9855
1991	99-781-64	A	G	A	5925	9856
1992	99-7840-281	A	C	S	5926	9857
1993	99-785-360	A	G	A	5927	9858
1994	99-7868-204	A	G	A	5928	9859
1995	99-7869-135	G	C	S	5929	9860
1996	99-7870-316	A	C	S	5930	9861
1997	99-7877-363	A	G	A	5931	9862
1998	99-7882-43	A	G	A	5932	9863
1999	99-7883-411	G	C	S	5933	9864
2000	99-7884-151	G	T	A	5934	9865
2001	99-7893-226	A	G	A	5935	9866
2002	99-7898-43	A	T	S	5936	9867
2003	99-7900-452	A	G	A	5937	9868
2004	99-791-236	C	T	S	5938	9869
2005	99-7917-429	A	C	S	5939	9870
2006	99-794-393	G	C	S	5940	9871
2007	99-7949-301	A	G	A	5941	9872
2008	99-795-211	C	T	S	5942	9873
2009	99-7967-152	C	T	S	5943	9874

2010	99-797-238	C	T	S	5944	9875
2011	99-7985-178	G	C	S	5945	9876
2012	99-7988-389	C	T	S	5946	9877
2013	99-8002-49	A	G	A	5947	9878
2014	99-8010-124	C	T	S	5948	9879
2015	99-8012-420	A	G	A	5949	9880
2016	99-8013-122	A	G	A	5950	9881
2017	99-8016-267	A	G	A	5951	9882
2018	99-8025-306	A	C	S	5952	9883
2019	99-8027-265	C	T	S	5953	9884
2020	99-8028-87	A	T	S	5954	9885
2021	99-8030-411	G	C	S	5955	9886
2022	99-8046-263	A	G	A	5956	9887
2023	99-8051-125	A	G	A	5957	9888
2024	99-806-152	C	T	S	5958	9889
2025	99-8063-174	C	T	S	5959	9890
2026	99-8067-79	A	G	A	5960	9891
2027	99-8068-258	C	T	S	5961	9892
2028	99-8081-340	C	T	S	5962	9893
2029	99-8088-247	A	C	S	5963	9894
2030	99-8089-246	A	G	A	5964	9895
2031	99-8095-164	G	T	A	5965	9896
2032	99-81-227	C	T	S	5966	9897
2033	99-810-117	A	G	A	5967	9898
2034	99-8102-124	C	T	S	5968	9899
2035	99-8120-354	A	G	A	5969	9900
2036	99-8128-302	C	T	S	5970	9901
2037	99-8141-65	A	C	S	5971	9902
2038	99-8161-230	A	G	A	5972	9903
2039	99-8162-210	C	T	S	5973	9904
2040	99-8164-397	A	G	S	5974	9905
2041	99-8170-163	C	T	S	5975	9906
2042	99-8173-352	C	T	S	5976	9907
2043	99-8181-228	A	T	S	5977	9908
2044	99-8186-76	G	T	A	5978	9909
2045	99-8188-369	G	T	A	5979	9910
2046	99-8192-168	C	T	S	5980	9911
2047	99-8219-373	A	G	A	5981	9912
2048	99-8245-192	G	C	S	5982	9913
2049	99-8255-365	A	G	A	5983	9914
2050	99-8256-148	A	G	A	5984	9915
2051	99-8266-393	A	G	A	5985	9916
2052	99-827-359	C	T	S	5986	9917
2053	99-8272-122	G	C	S	5987	9918
2054	99-8276-65	A	C	S	5988	9919
2055	99-8278-412	A	G	A	5989	9920
2056	99-8279-252	A	T	S	5990	9921
2057	99-828-259	C	T	S	5991	9922
2058	99-8287-122	A	C	S	5992	9923
2059	99-8289-179	G	C	S	5993	9924
2060	99-8290-174	A	G	A	5994	9925
2061	99-8292-240	A	G	A	5995	9926
2062	99-8294-408	A	G	A	5996	9927
2063	99-8308-237	A	C	S	5997	9928
2064	99-8313-107	A	G	A	5998	9929
2065	99-8318-50	A	G	A	5999	9930

2066	99-8328-298	C	T	S	6000	9931
2067	99-8338-212	G	C	S	6001	9932
2068	99-8340-364	C	T	S	6002	9933
2069	99-8341-99	A	G	A	6003	9934
2070	99-8342-33	A	C	S	6004	9935
2071	99-8353-291	A	C	S	6005	9936
2072	99-8360-401	C	T	S	6006	9937
2073	99-8361-103	C	T	S	6007	9938
2074	99-8367-239	C	T	S	6008	9939
2075	99-8369-276	G	T	A	6009	9940
2076	99-8377-429	C	T	S	6010	9941
2077	99-8378-69	A	G	A	6011	9942
2078	99-8379-337	C	T	S	6012	9943
2079	99-8381-114	C	T	S	6013	9944
2080	99-8383-158	G	T	A	6014	9945
2081	99-8385-244	A	G	A	6015	9946
2082	99-840-68	A	C	S	6016	9947
2083	99-8402-113	G	C	S	6017	9948
2084	99-8414-183	A	T	S	6018	9949
2085	99-8441-298	A	C	S	6019	9950
2086	99-8442-95	C	T	S	6020	9951
2087	99-8453-358	A	G	A	6021	9952
2088	99-8454-152	C	T	S	6022	9953
2089	99-8456-266	A	C	S	6023	9954
2090	99-8457-239	C	T	S	6024	9955
2091	99-8470-275	A	G	A	6025	9956
2092	99-8472-152	G	C	S	6026	9957
2093	99-8476-216	A	G	A	6027	9958
2094	99-8478-385	G	T	A	6028	9959
2095	99-8487-245	C	T	S	6029	9960
2096	99-8491-339	A	G	A	6030	9961
2097	99-8499-107	C	T	S	6031	9962
2098	99-8505-269	A	G	A	6032	9963
2099	99-851-237	C	T	S	6033	9964
2100	99-8510-44	A	C	S	6034	9965
2101	99-8514-434	C	T	S	6035	9966
2102	99-8530-209	A	G	A	6036	9967
2103	99-854-415	C	T	S	6037	9968
2104	99-8546-116	C	T	S	6038	9969
2105	99-8571-396	A	G	A	6039	9970
2106	99-8575-401	C	T	S	6040	9971
2107	99-8576-321	A	G	A	6041	9972
2108	99-8578-407	A	C	S	6042	9973
2109	99-8581-443	C	T	S	6043	9974
2110	99-8583-146	A	C	S	6044	9975
2111	99-8588-369	A	G	A	6045	9976
2112	99-8590-287	A	G	A	6046	9977
2113	99-860-419	C	T	S	6047	9978
2114	99-8600-393	A	T	S	6048	9979
2115	99-8609-434	G	T	A	6049	9980
2116	99-8611-383	A	G	A	6050	9981
2117	99-862-233	A	C	S	6051	9982
2118	99-8626-133	C	T	S	6052	9983
2119	99-8632-413	A	G	A	6053	9984
2120	99-8638-107	C	T	S	6054	9985
2121	99-8641-418	C	T	S	6055	9986

2122	99-8648-169	A	T	S	6056	9987
2123	99-8654-157	A	G	A	6057	9988
2124	99-8655-77	C	T	S	6058	9989
2125	99-8658-168	A	G	A	6059	9990
2126	99-866-160	C	T	S	6060	9991
2127	99-8662-192	A	C	S	6061	9992
2128	99-8663-39	C	T	S	6062	9993
2129	99-8665-182	A	C	S	6063	9994
2130	99-8671-143	A	G	A	6064	9995
2131	99-8695-147	A	T	S	6065	9996
2132	99-870-379	C	T	S	6066	9997
2133	99-8703-42	C	T	S	6067	9998
2134	99-8715-315	A	G	A	6068	9999
2135	99-8725-240	A	G	A	6069	10000
2136	99-8732-105	A	G	A	6070	10001
2137	99-8744-283	A	G	A	6071	10002
2138	99-8748-239	C	T	S	6072	10003
2139	99-8755-402	C	T	S	6073	10004
2140	99-8761-163	A	G	A	6074	10005
2141	99-8775-410	C	T	S	6075	10006
2142	99-8778-416	A	G	A	6076	10007
2143	99-8780-454	C	T	S	6077	10008
2144	99-8796-142	G	T	A	6078	10009
2145	99-8799-211	A	G	A	6079	10010
2146	99-88-216	A	G	S	6080	10011
2147	99-8800-250	C	T	S	6081	10012
2148	99-8802-119	A	G	A	6082	10013
2149	99-8804-83	A	C	S	6083	10014
2150	99-8812-220	A	G	A	6084	10015
2151	99-8827-400	C	T	S	6085	10016
2152	99-8831-41	C	T	S	6086	10017
2153	99-8835-400	A	G	A	6087	10018
2154	99-8849-167	A	G	A	6088	10019
2155	99-8857-96	A	T	S	6089	10020
2156	99-8866-150	A	G	S	6090	10021
2157	99-8867-278	A	T	S	6091	10022
2158	99-8872-391	A	T	S	6092	10023
2159	99-8885-447	A	G	A	6093	10024
2160	99-8887-397	A	G	A	6094	10025
2161	99-8894-123	A	G	A	6095	10026
2162	99-8895-272	A	T	S	6096	10027
2163	99-8901-283	A	G	A	6097	10028
2164	99-8905-184	G	C	S	6098	10029
2165	99-8910-170	C	T	S	6099	10030
2166	99-8923-138	C	T	S	6100	10031
2167	99-8924-415	A	T	S	6101	10032
2168	99-8960-426	A	C	S	6102	10033
2169	99-8963-409	A	C	S	6103	10034
2170	99-8974-386	C	T	S	6104	10035
2171	99-8978-52	A	G	A	6105	10036
2172	99-8992-43	A	G	A	6106	10037
2173	99-9015-255	A	G	A	6107	10038
2174	99-9020-110	G	T	A	6108	10039
2175	99-9026-273	A	G	A	6109	10040
2176	99-9029-132	A	G	A	6110	10041
2177	99-9047-183	A	G	A	6111	10042

2178	99-9053-311	A	C	S	6112	10043
2179	99-9059-197	A	T	S	6113	10044
2180	99-9061-309	A	G	A	6114	10045
2181	99-9064-194	A	G	A	6115	10046
2182	99-9079-158	A	G	A	6116	10047
2183	99-9084-200	A	G	A	6117	10048
2184	99-9092-167	A	G	A	6118	10049
2185	99-9097-342	A	G	A	6119	10050
2186	99-9105-68	G	T	A	6120	10051
2187	99-9118-393	C	T	S	6121	10052
2188	99-9120-197	C	T	S	6122	10053
2189	99-9126-25	G	C	S	6123	10054
2190	99-913-140	A	G	A	6124	10055
2191	99-9141-307	G	C	S	6125	10056
2192	99-9152-154	C	T	S	6126	10057
2193	99-9157-329	A	C	S	6127	10058
2194	99-9175-329	A	G	A	6128	10059
2195	99-9204-245	C	T	S	6129	10060
2196	99-921-285	C	T	S	6130	10061
2197	99-924-93	A	G	A	6131	10062
2198	99-9240-109	C	T	S	6132	10063
2199	99-9250-450	G	T	A	6133	10064
2200	99-9254-404	A	G	A	6134	10065
2201	99-926-98	G	T	A	6135	10066
2202	99-9263-283	A	G	A	6136	10067
2203	99-9271-70	G	T	A	6137	10068
2204	99-9274-246	C	T	S	6138	10069
2205	99-9276-163	C	T	S	6139	10070
2206	99-9355-134	C	T	S	6140	10071
2207	99-9368-223	A	G	A	6141	10072
2208	99-937-125	A	T	S	6142	10073
2209	99-9372-298	A	C	S	6143	10074
2210	99-9381-429	A	G	A	6144	10075
2211	99-9385-387	C	T	S	6145	10076
2212	99-9389-363	A	G	A	6146	10077
2213	99-9395-133	C	T	S	6147	10078
2214	99-9401-80	A	G	A	6148	10079
2215	99-9402-263	C	T	S	6149	10080
2216	99-9404-338	A	T	S	6150	10081
2217	99-9405-421	A	G	A	6151	10082
2218	99-941-265	A	T	S	6152	10083
2219	99-9410-205	C	T	S	6153	10084
2220	99-9412-202	C	T	S	6154	10085
2221	99-9417-151	C	T	S	6155	10086
2222	99-942-381	C	T	S	6156	10087
2223	99-9420-318	C	T	S	6157	10088
2224	99-9421-51	A	G	A	6158	10089
2225	99-9422-41	A	G	A	6159	10090
2226	99-9423-394	A	G	A	6160	10091
2227	99-9424-229	A	G	A	6161	10092
2228	99-9427-454	C	T	S	6162	10093
2229	99-9446-394	A	G	A	6163	10094
2230	99-9448-292	C	T	S	6164	10095
2231	99-9462-362	A	G	A	6165	10096
2232	99-9471-230	A	G	A	6166	10097
2233	99-949-214	A	G	A	6167	10098

2234	99-9491-388	A	G	A	6168	10099
2235	99-9493-455	G	C	S	6169	10100
2236	99-9499-111	A	T	S	6170	10101
2237	99-950-418	C	T	S	6171	10102
2238	99-9513-285	A	G	A	6172	10103
2239	99-952-252	G	C	S	6173	10104
2240	99-9527-211	C	T	S	6174	10105
2241	99-9531-340	C	T	S	6175	10106
2242	99-9538-395	A	G	A	6176	10107
2243	99-954-45	A	C	S	6177	10108
2244	99-9542-164	G	T	A	6178	10109
2245	99-9545-100	A	C	S	6179	10110
2246	99-9554-345	A	C	S	6180	10111
2247	99-9555-348	C	T	S	6181	10112
2248	99-9556-349	A	G	A	6182	10113
2249	99-9567-229	C	T	A	6183	10114
2250	99-9572-240	C	T	A	6184	10115
2251	99-9577-284	C	T	A	6185	10116
2252	99-9579-363	G	C	S	6186	10117
2253	99-958-92	C	T	A	6187	10118
2254	99-9587-338	A	G	S	6188	10119
2255	99-961-150	C	T	S	6189	10120
2256	99-963-395	A	G	A	6190	10121
2257	99-965-165	C	T	S	6191	10122
2258	99-967-306	C	T	S	6192	10123
2259	99-976-246	C	T	S	6193	10124
2260	99-979-343	A	C	S	6194	10125
2261	99-10000-518	G	A	S	6195	10126
2262	99-10016-115	T	A	S	6196	10127
2263	99-10027-378	G	A	S	6197	10128
2264	99-10028-93	C	G	S	6198	10129
2265	99-10031-130	T	C	A	6199	10130
2266	99-10046-199	T	C	S	6200	10131
2267	99-10064-252	T	C	S	6201	10132
2268	99-10066-465	G	T	A	6202	10133
2269	99-10067-168	G	A	A	6203	10134
2270	99-10078-341	T	C	S	6204	10135
2271	99-10104-464	C	A	S	6205	10136
2272	99-10106-247	G	T	A	6206	10137
2273	99-10108-419	G	A	S	6207	10138
2274	99-10118-323	T	C	A	6208	10139
2275	99-10126-413	C	T	A	6209	10140
2276	99-10127-506	A	T	S	6210	10141
2277	99-10137-195	T	G	A	6211	10142
2278	99-10142-293	G	A	S	6212	10143
2279	99-10143-111	A	G	S	6213	10144
2280	99-10146-202	T	A	S	6214	10145
2281	99-10149-291	T	C	A	6215	10146
2282	99-10151-340	G	A	S	6216	10147
2283	99-10153-267	A	G	S	6217	10148
2284	99-10155-423	C	G	S	6218	10149
2285	99-10173-122	A	G	S	6219	10150
2286	99-10179-48	G	A	S	6220	10151
2287	99-1018-244	A	C	A	6221	10152
2288	99-10183-166	G	T	A	6222	10153
2289	99-10185-402	C	A	S	6223	10154

2290	99-10188-116	G	A	S	6224	10155
2291	99-10201-115	C	T	A	6225	10156
2292	99-10207-173	A	G	S	6226	10157
2293	99-10211-380	A	G	S	6227	10158
2294	99-10216-336	A	C	S	6228	10159
2295	99-10220-312	C	A	S	6229	10160
2296	99-10223-153	T	A	S	6230	10161
2297	99-10224-223	T	A	S	6231	10162
2298	99-10234-334	A	G	S	6232	10163
2299	99-1024-403	G	A	S	6233	10164
2300	99-10245-197	A	G	S	6234	10165
2301	99-10256-41	A	G	S	6235	10166
2302	99-10264-82	G	A	S	6236	10167
2303	99-10266-290	T	A	S	6237	10168
2304	99-10267-409	A	C	S	6238	10169
2305	99-10303-406	C	T	A	6239	10170
2306	99-10304-88	A	G	S	6240	10171
2307	99-10312-155	C	T	A	6241	10172
2308	99-10318-230	C	T	A	6242	10173
2309	99-10330-432	G	A	S	6243	10174
2310	99-10332-89	C	T	A	6244	10175
2311	99-10345-182	T	G	A	6245	10176
2312	99-10353-285	G	T	A	6246	10177
2313	99-10364-331	A	G	S	6247	10178
2314	99-10369-41	T	C	A	6248	10179
2315	99-10374-343	C	T	A	6249	10180
2316	99-10381-328	T	A	S	6250	10181
2317	99-10389-114	G	T	A	6251	10182
2318	99-10390-172	A	G	S	6252	10183
2319	99-10414-128	C	T	A	6253	10184
2320	99-10434-121	G	T	S	6254	10185
2321	99-10436-162	C	T	A	6255	10186
2322	99-10438-281	C	A	S	6256	10187
2323	99-10446-425	G	C	S	6257	10188
2324	99-10451-188	A	T	S	6258	10189
2325	99-10452-306	A	G	S	6259	10190
2326	99-10457-310	T	C	A	6260	10191
2327	99-10470-405	A	C	S	6261	10192
2328	99-10471-88	C	T	A	6262	10193
2329	99-10473-259	T	C	A	6263	10194
2330	99-10474-223	G	C	S	6264	10195
2331	99-10481-217	C	T	A	6265	10196
2332	99-10487-57	T	C	A	6266	10197
2333	99-10488-146	G	C	S	6267	10198
2334	99-10491-300	C	T	A	6268	10199
2335	99-10499-102	T	G	A	6269	10200
2336	99-10502-161	T	C	A	6270	10201
2337	99-10506-307	A	C	S	6271	10202
2338	99-10507-216	C	T	A	6272	10203
2339	99-10509-122	T	G	A	6273	10204
2340	99-1051-284	G	A	A	6274	10205
2341	99-10513-347	T	C	A	6275	10206
2342	99-10514-546	G	T	A	6276	10207
2343	99-10521-296	C	T	A	6277	10208
2344	99-10522-395	A	C	A	6278	10209
2345	99-10536-90	C	A	S	6279	10210

2346	99-10539-208	G	A	S	6280	10211
2347	99-10542-326	C	T	A	6281	10212
2348	99-10543-278	A	T	S	6282	10213
2349	99-1055-140	C	T	S	6283	10214
2350	99-10557-276	T	C	A	6284	10215
2351	99-10567-233	A	G	S	6285	10216
2352	99-10570-107	G	A	S	6286	10217
2353	99-10573-375	G	A	S	6287	10218
2354	99-10575-416	T	C	A	6288	10219
2355	99-10576-351	A	G	S	6289	10220
2356	99-10577-36	T	C	A	6290	10221
2357	99-10581-354	G	C	S	6291	10222
2358	99-10589-360	A	C	S	6292	10223
2359	99-10601-463	C	G	S	6293	10224
2360	99-10606-92	G	A	S	6294	10225
2361	99-10608-353	C	T	A	6295	10226
2362	99-10613-277	C	G	S	6296	10227
2363	99-10618-404	A	T	S	6297	10228
2364	99-10626-196	G	C	S	6298	10229
2365	99-10630-236	G	A	S	6299	10230
2366	99-10632-55	A	T	S	6300	10231
2367	99-10634-141	G	A	S	6301	10232
2368	99-10643-161	T	C	A	6302	10233
2369	99-10659-208	C	T	A	6303	10234
2370	99-10661-153	G	A	S	6304	10235
2371	99-10662-397	C	T	A	6305	10236
2372	99-10667-251	G	C	S	6306	10237
2373	99-10675-109	A	G	S	6307	10238
2374	99-1068-309	C	T	S	6308	10239
2375	99-10683-117	A	G	S	6309	10240
2376	99-10689-419	A	G	S	6310	10241
2377	99-10692-377	G	A	S	6311	10242
2378	99-10694-446	C	T	A	6312	10243
2379	99-10695-161	C	T	A	6313	10244
2380	99-1070-342	C	T	S	6314	10245
2381	99-10702-261	G	A	S	6315	10246
2382	99-10706-228	T	C	A	6316	10247
2383	99-10708-28	G	A	S	6317	10248
2384	99-10709-460	G	A	S	6318	10249
2385	99-10715-43	A	G	S	6319	10250
2386	99-10719-455	C	T	S	6320	10251
2387	99-10720-63	A	G	S	6321	10252
2388	99-10731-195	C	T	S	6322	10253
2389	99-10735-238	T	C	A	6323	10254
2390	99-1074-127	G	A	A	6324	10255
2391	99-10741-421	A	C	S	6325	10256
2392	99-10743-315	T	A	S	6326	10257
2393	99-1075-314	G	A	A	6327	10258
2394	99-10752-366	A	G	S	6328	10259
2395	99-1076-116	C	T	A	6329	10260
2396	99-10769-291	A	C	S	6330	10261
2397	99-10771-266	A	C	S	6331	10262
2398	99-10775-331	A	G	S	6332	10263
2399	99-10776-447	T	A	S	6333	10264
2400	99-1079-237	C	G	S	6334	10265
2401	99-1081-159	A	T	S	6335	10266

2402	99-10816-272	G	A	S	6336	10267
2403	99-1082-180	T	A	S	6337	10268
2404	99-10839-239	C	T	A	6338	10269
2405	99-10842-232	A	G	S	6339	10270
2406	99-10843-114	A	G	S	6340	10271
2407	99-10856-246	C	G	S	6341	10272
2408	99-10861-96	T	C	A	6342	10273
2409	99-10862-397	T	C	A	6343	10274
2410	99-10864-418	C	G	S	6344	10275
2411	99-10870-234	G	A	S	6345	10276
2412	99-10874-69	G	A	S	6346	10277
2413	99-10879-386	A	G	S	6347	10278
2414	99-10887-214	A	G	S	6348	10279
2415	99-10890-201	T	G	A	6349	10280
2416	99-10894-35	T	C	A	6350	10281
2417	99-10898-209	G	T	A	6351	10282
2418	99-10904-111	C	T	A	6352	10283
2419	99-10905-85	C	T	A	6353	10284
2420	99-1091-446	C	T	S	6354	10285
2421	99-10927-388	G	C	S	6355	10286
2422	99-10929-298	T	A	S	6356	10287
2423	99-10930-95	A	T	S	6357	10288
2424	99-10937-64	T	C	A	6358	10289
2425	99-10944-83	G	A	S	6359	10290
2426	99-10951-434	T	C	A	6360	10291
2427	99-10959-113	C	T	A	6361	10292
2428	99-10964-89	T	C	A	6362	10293
2429	99-10965-174	C	A	S	6363	10294
2430	99-10966-113	G	A	S	6364	10295
2431	99-10974-193	T	A	S	6365	10296
2432	99-10978-393	T	A	S	6366	10297
2433	99-10979-156	T	A	S	6367	10298
2434	99-10988-242	G	A	S	6368	10299
2435	99-10992-98	G	A	S	6369	10300
2436	99-11000-163	A	G	S	6370	10301
2437	99-11001-393	C	T	A	6371	10302
2438	99-11003-361	G	A	S	6372	10303
2439	99-11006-426	C	T	A	6373	10304
2440	99-11007-68	T	C	A	6374	10305
2441	99-11014-194	C	A	S	6375	10306
2442	99-11034-317	C	T	A	6376	10307
2443	99-11035-299	C	T	A	6377	10308
2444	99-11037-218	C	A	S	6378	10309
2445	99-1105-127	A	C	S	6379	10310
2446	99-11051-154	A	T	S	6380	10311
2447	99-11063-111	G	C	S	6381	10312
2448	99-11074-187	A	G	S	6382	10313
2449	99-11075-311	G	A	S	6383	10314
2450	99-11089-424	C	A	S	6384	10315
2451	99-11094-427	G	A	A	6385	10316
2452	99-11099-179	A	G	S	6386	10317
2453	99-11103-88	A	T	S	6387	10318
2454	99-11106-117	C	T	A	6388	10319
2455	99-11110-375	C	T	A	6389	10320
2456	99-11115-133	A	G	S	6390	10321
2457	99-11119-132	T	C	A	6391	10322

2458	99-11128-162	A	G	S	6392	10323
2459	99-11136-374	G	A	S	6393	10324
2460	99-11142-139	T	C	A	6394	10325
2461	99-11143-443	A	C	S	6395	10326
2462	99-11144-137	G	A	S	6396	10327
2463	99-11148-369	A	C	S	6397	10328
2464	99-11158-255	C	T	A	6398	10329
2465	99-11163-293	C	G	S	6399	10330
2466	99-11164-298	C	T	A	6400	10331
2467	99-11168-197	G	A	S	6401	10332
2468	99-11175-348	A	C	S	6402	10333
2469	99-11179-239	C	T	A	6403	10334
2470	99-11180-148	A	G	S	6404	10335
2471	99-11183-166	G	A	S	6405	10336
2472	99-11191-86	A	G	S	6406	10337
2473	99-11210-235	G	A	S	6407	10338
2474	99-11214-188	T	G	A	6408	10339
2475	99-11218-174	G	A	S	6409	10340
2476	99-11236-63	C	T	A	6410	10341
2477	99-11247-86	C	G	S	6411	10342
2478	99-11248-404	A	G	S	6412	10343
2479	99-11252-263	T	A	S	6413	10344
2480	99-11255-375	G	A	S	6414	10345
2481	99-11260-422	C	T	A	6415	10346
2482	99-11261-255	G	A	S	6416	10347
2483	99-11293-125	G	A	S	6417	10348
2484	99-11313-95	G	A	S	6418	10349
2485	99-11320-29	C	T	A	6419	10350
2486	99-11326-356	A	G	S	6420	10351
2487	99-11340-89	C	T	A	6421	10352
2488	99-11346-222	T	C	A	6422	10353
2489	99-11350-116	A	G	S	6423	10354
2490	99-11356-187	A	T	S	6424	10355
2491	99-11362-334	C	T	A	6425	10356
2492	99-11369-112	C	G	S	6426	10357
2493	99-11372-162	C	A	S	6427	10358
2494	99-11377-384	A	G	S	6428	10359
2495	99-11381-256	C	G	S	6429	10360
2496	99-11385-245	C	T	A	6430	10361
2497	99-11413-239	A	G	S	6431	10362
2498	99-1143-340	G	A	S	6432	10363
2499	99-11430-162	C	A	S	6433	10364
2500	99-11431-333	A	G	S	6434	10365
2501	99-11449-297	A	G	S	6435	10366
2502	99-11464-236	C	G	S	6436	10367
2503	99-11466-107	T	C	A	6437	10368
2504	99-11485-396	T	A	S	6438	10369
2505	99-11492-360	A	T	S	6439	10370
2506	99-11499-45	G	A	S	6440	10371
2507	99-11505-92	A	G	S	6441	10372
2508	99-11506-224	G	C	S	6442	10373
2509	99-11520-170	A	G	S	6443	10374
2510	99-11521-146	G	T	A	6444	10375
2511	99-11522-313	C	T	A	6445	10376
2512	99-11528-137	A	T	S	6446	10377
2513	99-11530-388	G	A	S	6447	10378

2514	99-11533-375	C	G	S	6448	10379
2515	99-11535-193	G	T	A	6449	10380
2516	99-11543-415	A	T	S	6450	10381
2517	99-11545-180	T	C	A	6451	10382
2518	99-11555-397	C	T	A	6452	10383
2519	99-11559-81	C	A	S	6453	10384
2520	99-11563-183	A	G	S	6454	10385
2521	99-11565-305	C	A	S	6455	10386
2522	99-11566-385	G	A	S	6456	10387
2523	99-11580-97	A	G	S	6457	10388
2524	99-11584-69	A	G	S	6458	10389
2525	99-11587-202	A	G	S	6459	10390
2526	99-11592-297	C	A	S	6460	10391
2527	99-11600-48	A	G	S	6461	10392
2528	99-11601-441	C	T	A	6462	10393
2529	99-11602-93	G	A	S	6463	10394
2530	99-11604-396	T	C	A	6464	10395
2531	99-11611-259	A	G	S	6465	10396
2532	99-11613-315	T	C	A	6466	10397
2533	99-11620-149	A	C	S	6467	10398
2534	99-11635-363	C	T	A	6468	10399
2535	99-11643-378	C	T	A	6469	10400
2536	99-11645-157	C	T	A	6470	10401
2537	99-11658-275	G	A	S	6471	10402
2538	99-11668-308	T	C	A	6472	10403
2539	99-11669-394	C	T	A	6473	10404
2540	99-11670-486	G	T	A	6474	10405
2541	99-11685-200	T	C	A	6475	10406
2542	99-11697-345	C	T	A	6476	10407
2543	99-11700-326	T	C	A	6477	10408
2544	99-11704-23	T	C	A	6478	10409
2545	99-11705-302	G	T	A	6479	10410
2546	99-11723-211	A	T	S	6480	10411
2547	99-11743-233	C	T	A	6481	10412
2548	99-11745-256	A	G	S	6482	10413
2549	99-11746-238	A	G	S	6483	10414
2550	99-11780-292	G	T	A	6484	10415
2551	99-11785-167	A	G	S	6485	10416
2552	99-11786-98	G	T	A	6486	10417
2553	99-11787-281	G	A	S	6487	10418
2554	99-11788-69	G	A	S	6488	10419
2555	99-11789-348	A	C	S	6489	10420
2556	99-11797-147	A	G	S	6490	10421
2557	99-11810-289	A	C	S	6491	10422
2558	99-11811-158	A	G	S	6492	10423
2559	99-1182-310	G	A	A	6493	10424
2560	99-11823-118	G	A	S	6494	10425
2561	99-11824-90	T	A	S	6495	10426
2562	99-1183-182	G	A	A	6496	10427
2563	99-11830-334	C	G	S	6497	10428
2564	99-11831-321	C	T	A	6498	10429
2565	99-11839-223	C	T	A	6499	10430
2566	99-11842-197	T	C	A	6500	10431
2567	99-1185-317	T	G	A	6501	10432
2568	99-11851-45	C	G	S	6502	10433
2569	99-11857-368	G	A	S	6503	10434

2570	99-1186-249	G	A	A	6504	10435
2571	99-11861-254	C	T	A	6505	10436
2572	99-11877-237	T	C	A	6506	10437
2573	99-11880-90	G	C	S	6507	10438
2574	99-11882-120	A	G	S	6508	10439
2575	99-11894-470	G	A	S	6509	10440
2576	99-11917-129	A	G	S	6510	10441
2577	99-11922-206	G	A	S	6511	10442
2578	99-11930-395	C	T	A	6512	10443
2579	99-11966-288	A	G	S	6513	10444
2580	99-11989-233	C	A	S	6514	10445
2581	99-11993-468	C	T	A	6515	10446
2582	99-12000-355	T	C	A	6516	10447
2583	99-12005-282	C	T	A	6517	10448
2584	99-12017-203	G	T	A	6518	10449
2585	99-1202-340	C	T	S	6519	10450
2586	99-12028-121	T	C	A	6520	10451
2587	99-1203-272	A	G	A	6521	10452
2588	99-12038-420	C	T	A	6522	10453
2589	99-12039-389	C	T	A	6523	10454
2590	99-12048-300	A	G	S	6524	10455
2591	99-12049-245	A	G	S	6525	10456
2592	99-12050-459	A	G	S	6526	10457
2593	99-12061-211	A	G	S	6527	10458
2594	99-12062-94	G	A	S	6528	10459
2595	99-12068-348	T	C	A	6529	10460
2596	99-12087-45	C	T	A	6530	10461
2597	99-1211-59	C	T	A	6531	10462
2598	99-12130-72	G	A	S	6532	10463
2599	99-12133-294	T	C	A	6533	10464
2600	99-12135-288	C	A	S	6534	10465
2601	99-12152-332	A	G	S	6535	10466
2602	99-12158-148	A	G	S	6536	10467
2603	99-12168-256	C	T	A	6537	10468
2604	99-12171-93	G	A	S	6538	10469
2605	99-12178-423	C	T	A	6539	10470
2606	99-12181-226	T	C	A	6540	10471
2607	99-12186-229	C	T	A	6541	10472
2608	99-12198-289	A	G	S	6542	10473
2609	99-12199-246	T	C	A	6543	10474
2610	99-12203-356	C	G	S	6544	10475
2611	99-12224-368	A	G	S	6545	10476
2612	99-12228-184	C	T	A	6546	10477
2613	99-12241-380	T	G	A	6547	10478
2614	99-12253-145	T	C	A	6548	10479
2615	99-12265-324	A	T	S	6549	10480
2616	99-12267-161	G	T	A	6550	10481
2617	99-12268-54	G	A	S	6551	10482
2618	99-12270-408	T	G	A	6552	10483
2619	99-12271-298	G	A	S	6553	10484
2620	99-12275-214	A	T	S	6554	10485
2621	99-12299-433	T	G	A	6555	10486
2622	99-12303-460	C	T	A	6556	10487
2623	99-12335-394	C	T	A	6557	10488
2624	99-12338-83	T	C	A	6558	10489
2625	99-12344-171	G	C	S	6559	10490

2626	99-12347-490	G	C	S	6560	10491
2627	99-12348-74	T	G	A	6561	10492
2628	99-12352-124	T	G	A	6562	10493
2629	99-12356-272	T	C	A	6563	10494
2630	99-12361-88	T	C	A	6564	10495
2631	99-12368-335	A	C	S	6565	10496
2632	99-12370-67	G	A	S	6566	10497
2633	99-12384-135	G	A	S	6567	10498
2634	99-12388-466	G	A	S	6568	10499
2635	99-12393-326	A	G	S	6569	10500
2636	99-12399-180	C	T	A	6570	10501
2637	99-12412-381	T	C	A	6571	10502
2638	99-12415-509	A	G	S	6572	10503
2639	99-12444-400	A	G	S	6573	10504
2640	99-12465-227	G	A	A	6574	10505
2641	99-12468-236	G	T	A	6575	10506
2642	99-12470-288	G	A	A	6576	10507
2643	99-12522-196	T	C	S	6577	10508
2644	99-12561-278	C	G	S	6578	10509
2645	99-12570-265	G	A	A	6579	10510
2646	99-12595-313	C	T	S	6580	10511
2647	99-12596-334	T	C	S	6581	10512
2648	99-12598-191	C	A	S	6582	10513
2649	99-12602-212	G	T	A	6583	10514
2650	99-12605-365	C	T	S	6584	10515
2651	99-12607-384	A	G	A	6585	10516
2652	99-12664-222	C	A	S	6586	10517
2653	99-12696-116	T	C	S	6587	10518
2654	99-12960-443	G	A	S	6588	10519
2655	99-12965-451	C	T	A	6589	10520
2656	99-12969-128	C	T	A	6590	10521
2657	99-12970-339	A	G	S	6591	10522
2658	99-12973-162	G	A	S	6592	10523
2659	99-13074-132	T	C	A	6593	10524
2660	99-13077-340	C	T	A	6594	10525
2661	99-1311-59	C	G	S	6595	10526
2662	99-13113-234	G	A	S	6596	10527
2663	99-13205-67	T	A	S	6597	10528
2664	99-1326-203	C	T	A	6598	10529
2665	99-1333-123	C	G	S	6599	10530
2666	99-1335-195	A	G	A	6600	10531
2667	99-13350-376	T	G	A	6601	10532
2668	99-13376-288	A	T	S	6602	10533
2669	99-13473-135	C	T	A	6603	10534
2670	99-13530-325	T	C	S	6604	10535
2671	99-13563-83	C	T	S	6605	10536
2672	99-13579-242	G	C	S	6606	10537
2673	99-13609-327	T	C	A	6607	10538
2674	99-13621-358	T	C	S	6608	10539
2675	99-1370-401	A	G	S	6609	10540
2676	99-13864-64	G	T	A	6610	10541
2677	99-13938-286	T	C	A	6611	10542
2678	99-13943-247	T	C	A	6612	10543
2679	99-13948-182	T	A	S	6613	10544
2680	99-13966-334	T	C	A	6614	10545
2681	99-14002-395	C	G	S	6615	10546

2682	99-14022-347	C	T	A	6616	10547
2683	99-14042-464	G	A	S	6617	10548
2684	99-14045-353	T	C	A	6618	10549
2685	99-14074-326	C	T	A	6619	10550
2686	99-14093-333	T	C	A	6620	10551
2687	99-14105-357	G	C	S	6621	10552
2688	99-14107-175	A	G	S	6622	10553
2689	99-14111-346	C	G	S	6623	10554
2690	99-14177-226	A	G	S	6624	10555
2691	99-14198-374	T	G	A	6625	10556
2692	99-14225-345	T	C	A	6626	10557
2693	99-14228-387	C	G	S	6627	10558
2694	99-14410-373	T	C	A	6628	10559
2695	99-14413-383	T	G	A	6629	10560
2696	99-14415-106	C	T	A	6630	10561
2697	99-14424-353	G	A	S	6631	10562
2698	99-14473-243	A	C	S	6632	10563
2699	99-14476-377	T	G	A	6633	10564
2700	99-14481-386	T	C	A	6634	10565
2701	99-14489-415	G	T	A	6635	10566
2702	99-14673-334	A	G	S	6636	10567
2703	99-14705-290	A	G	S	6637	10568
2704	99-14739-205	C	G	S	6638	10569
2705	99-14743-418	C	T	A	6639	10570
2706	99-14944-119	A	C	S	6640	10571
2707	99-14949-472	G	A	S	6641	10572
2708	99-15000-259	C	T	A	6642	10573
2709	99-15067-278	T	C	A	6643	10574
2710	99-15192-224	C	T	A	6644	10575
2711	99-15369-90	C	T	A	6645	10576
2712	99-15423-223	G	A	S	6646	10577
2713	99-15471-316	G	T	A	6647	10578
2714	99-15538-250	T	C	A	6648	10579
2715	99-15588-430	A	G	S	6649	10580
2716	99-15615-368	G	A	S	6650	10581
2717	99-15653-359	G	A	S	6651	10582
2718	99-15654-122	G	A	S	6652	10583
2719	99-15724-147	C	T	A	6653	10584
2720	99-15784-28	G	T	A	6654	10585
2721	99-1591-235	G	A	A	6655	10586
2722	99-15963-394	A	G	S	6656	10587
2723	99-15984-100	A	G	S	6657	10588
2724	99-16017-426	C	T	A	6658	10589
2725	99-16026-359	G	A	S	6659	10590
2726	99-1624-377	G	A	A	6660	10591
2727	99-16241-126	T	G	A	6661	10592
2728	99-16259-304	A	C	S	6662	10593
2729	99-16284-389	T	C	A	6663	10594
2730	99-16343-30	T	C	A	6664	10595
2731	99-16401-88	C	T	A	6665	10596
2732	99-16406-349	C	T	A	6666	10597
2733	99-16422-240	A	G	S	6667	10598
2734	99-16428-275	C	T	A	6668	10599
2735	99-16430-358	T	G	A	6669	10600
2736	99-16432-114	T	A	S	6670	10601
2737	99-16647-382	T	C	A	6671	10602

2738	99-16661-147	G	A	S	6672	10603
2739	99-16686-82	A	C	S	6673	10604
2740	99-16714-82	G	A	A	6674	10605
2741	99-16735-210	A	G	A	6675	10606
2742	99-16739-245	G	A	A	6676	10607
2743	99-16751-318	A	T	S	6677	10608
2744	99-16752-78	C	G	S	6678	10609
2745	99-16754-63	A	G	A	6679	10610
2746	99-16758-60	G	T	A	6680	10611
2747	99-16761-370	A	G	A	6681	10612
2748	99-16769-459	G	A	A	6682	10613
2749	99-16771-222	C	A	S	6683	10614
2750	99-16772-36	T	C	S	6684	10615
2751	99-16774-183	G	T	A	6685	10616
2752	99-16776-275	C	G	S	6686	10617
2753	99-16794-291	A	T	S	6687	10618
2754	99-16797-385	G	A	A	6688	10619
2755	99-16815-282	G	A	A	6689	10620
2756	99-16835-413	G	A	A	6690	10621
2757	99-16909-151	T	C	A	6691	10622
2758	99-17024-215	C	T	S	6692	10623
2759	99-17046-162	A	C	S	6693	10624
2760	99-17075-173	C	T	A	6694	10625
2761	99-17107-271	G	T	A	6695	10626
2762	99-17162-81	G	T	A	6696	10627
2763	99-17167-55	A	G	S	6697	10628
2764	99-17214-451	T	C	A	6698	10629
2765	99-17254-339	G	A	A	6699	10630
2766	99-17402-339	G	A	S	6700	10631
2767	99-17492-271	T	C	A	6701	10632
2768	99-17519-116	G	A	S	6702	10633
2769	99-17581-374	G	A	S	6703	10634
2770	99-17716-400	C	T	S	6704	10635
2771	99-18122-403	C	T	A	6705	10636
2772	99-18126-160	A	T	S	6706	10637
2773	99-18127-283	A	G	S	6707	10638
2774	99-18141-152	T	C	A	6708	10639
2775	99-18190-317	G	A	S	6709	10640
2776	99-18321-371	T	C	S	6710	10641
2777	99-1833-56	T	C	S	6711	10642
2778	99-18334-485	A	G	A	6712	10643
2779	99-18396-324	A	C	S	6713	10644
2780	99-18471-410	A	G	A	6714	10645
2781	99-18528-195	G	A	A	6715	10646
2782	99-18576-182	A	C	S	6716	10647
2783	99-18581-34	T	C	S	6717	10648
2784	99-18645-309	G	A	S	6718	10649
2785	99-18696-213	T	C	S	6719	10650
2786	99-18698-346	C	G	S	6720	10651
2787	99-18710-208	C	T	S	6721	10652
2788	99-18717-319	T	A	S	6722	10653
2789	99-18718-362	C	G	S	6723	10654
2790	99-18771-300	C	T	S	6724	10655
2791	99-1879-393	G	A	A	6725	10656
2792	99-18886-50	T	A	S	6726	10657
2793	99-18944-242	C	T	S	6727	10658

2794	99-19023-347	T	C	S	6728	10659
2795	99-19027-222	A	G	A	6729	10660
2796	99-19033-208	G	T	A	6730	10661
2797	99-19040-395	A	G	A	6731	10662
2798	99-19041-87	A	G	A	6732	10663
2799	99-19048-487	C	T	S	6733	10664
2800	99-19050-251	T	C	S	6734	10665
2801	99-19053-241	T	A	S	6735	10666
2802	99-19055-264	T	C	S	6736	10667
2803	99-19059-347	C	A	S	6737	10668
2804	99-19069-44	C	T	S	6738	10669
2805	99-19095-106	T	C	S	6739	10670
2806	99-19096-317	A	G	A	6740	10671
2807	99-19104-66	T	C	S	6741	10672
2808	99-19105-114	A	G	A	6742	10673
2809	99-19108-156	C	T	S	6743	10674
2810	99-19110-175	A	G	A	6744	10675
2811	99-19122-58	A	G	A	6745	10676
2812	99-19123-242	A	G	A	6746	10677
2813	99-19130-86	T	C	S	6747	10678
2814	99-19137-156	C	T	S	6748	10679
2815	99-19142-245	T	C	S	6749	10680
2816	99-19154-146	C	T	S	6750	10681
2817	99-19155-75	A	G	A	6751	10682
2818	99-19167-269	A	G	A	6752	10683
2819	99-19170-193	A	G	A	6753	10684
2820	99-19171-120	G	A	S	6754	10685
2821	99-19175-150	G	A	A	6755	10686
2822	99-19177-425	C	T	S	6756	10687
2823	99-19178-163	T	C	S	6757	10688
2824	99-19210-502	A	G	A	6758	10689
2825	99-19219-316	G	A	A	6759	10690
2826	99-19220-220	T	C	S	6760	10691
2827	99-19223-238	C	T	S	6761	10692
2828	99-19226-169	A	G	A	6762	10693
2829	99-19228-319	G	A	A	6763	10694
2830	99-19236-409	G	A	A	6764	10695
2831	99-19241-362	C	T	S	6765	10696
2832	99-19242-254	G	A	A	6766	10697
2833	99-19283-172	A	G	A	6767	10698
2834	99-19295-95	C	T	S	6768	10699
2835	99-19304-270	T	C	S	6769	10700
2836	99-19305-367	A	C	S	6770	10701
2837	99-19309-296	T	C	S	6771	10702
2838	99-19312-34	G	A	A	6772	10703
2839	99-19324-214	A	G	A	6773	10704
2840	99-19330-274	G	T	A	6774	10705
2841	99-19347-228	G	A	A	6775	10706
2842	99-19348-229	T	C	S	6776	10707
2843	99-19351-360	A	T	S	6777	10708
2844	99-19368-92	C	A	S	6778	10709
2845	99-19375-434	G	A	A	6779	10710
2846	99-19381-249	G	A	A	6780	10711
2847	99-19383-432	G	C	S	6781	10712
2848	99-19384-63	G	A	A	6782	10713
2849	99-19418-61	G	A	A	6783	10714

2850	99-19420-86	A	T	S	6784	10715
2851	99-19426-250	G	A	A	6785	10716
2852	99-19431-249	A	G	A	6786	10717
2853	99-19438-261	T	A	S	6787	10718
2854	99-19442-48	G	A	A	6788	10719
2855	99-19444-350	T	C	S	6789	10720
2856	99-19450-440	C	A	S	6790	10721
2857	99-19453-250	G	T	A	6791	10722
2858	99-19457-182	T	C	S	6792	10723
2859	99-19460-346	G	T	A	6793	10724
2860	99-19461-282	T	C	S	6794	10725
2861	99-19464-165	A	G	A	6795	10726
2862	99-19466-406	C	T	S	6796	10727
2863	99-19474-266	G	T	S	6797	10728
2864	99-19475-113	G	C	S	6798	10729
2865	99-19477-208	T	C	S	6799	10730
2866	99-19504-468	T	C	S	6800	10731
2867	99-19528-278	C	T	S	6801	10732
2868	99-19529-118	A	G	A	6802	10733
2869	99-19532-207	G	T	A	6803	10734
2870	99-19538-272	A	G	A	6804	10735
2871	99-19544-329	G	A	A	6805	10736
2872	99-19546-473	G	A	A	6806	10737
2873	99-19550-397	G	T	A	6807	10738
2874	99-19553-52	T	C	S	6808	10739
2875	99-19557-152	A	G	A	6809	10740
2876	99-19560-289	G	T	A	6810	10741
2877	99-19562-227	G	A	A	6811	10742
2878	99-19566-337	C	G	S	6812	10743
2879	99-19568-273	C	T	S	6813	10744
2880	99-19575-299	A	G	A	6814	10745
2881	99-19578-307	A	G	A	6815	10746
2882	99-19580-323	C	T	S	6816	10747
2883	99-19584-352	C	T	S	6817	10748
2884	99-19588-438	G	A	A	6818	10749
2885	99-19589-118	C	T	S	6819	10750
2886	99-19601-95	T	G	A	6820	10751
2887	99-19624-48	C	G	S	6821	10752
2888	99-19634-149	A	G	A	6822	10753
2889	99-19639-225	A	G	A	6823	10754
2890	99-19645-339	A	G	A	6824	10755
2891	99-19650-338	G	C	S	6825	10756
2892	99-19651-133	A	C	S	6826	10757
2893	99-19664-328	G	A	A	6827	10758
2894	99-19673-125	C	G	S	6828	10759
2895	99-19678-269	G	C	S	6829	10760
2896	99-19685-39	A	T	S	6830	10761
2897	99-19697-304	C	T	S	6831	10762
2898	99-19703-75	C	G	S	6832	10763
2899	99-19705-128	T	C	S	6833	10764
2900	99-19709-299	T	G	A	6834	10765
2901	99-19711-169	G	T	A	6835	10766
2902	99-19722-150	T	C	S	6836	10767
2903	99-19731-244	A	G	A	6837	10768
2904	99-19732-385	T	A	S	6838	10769
2905	99-19736-62	G	A	A	6839	10770

2906	99-19745-330	C	T	S	6840	10771
2907	99-19749-158	G	A	A	6841	10772
2908	99-19752-88	T	C	S	6842	10773
2909	99-19753-300	G	A	A	6843	10774
2910	99-19756-85	T	C	S	6844	10775
2911	99-19764-177	T	C	S	6845	10776
2912	99-19769-227	C	T	S	6846	10777
2913	99-19780-179	A	G	A	6847	10778
2914	99-19785-140	A	G	A	6848	10779
2915	99-19790-398	G	A	A	6849	10780
2916	99-19791-103	G	T	A	6850	10781
2917	99-19795-199	A	G	A	6851	10782
2918	99-19796-256	T	G	A	6852	10783
2919	99-19807-396	C	T	S	6853	10784
2920	99-19813-55	C	T	S	6854	10785
2921	99-19818-156	C	T	S	6855	10786
2922	99-19826-285	G	A	A	6856	10787
2923	99-19839-223	A	G	A	6857	10788
2924	99-19851-40	C	G	S	6858	10789
2925	99-19858-91	C	T	S	6859	10790
2926	99-19860-68	A	G	A	6860	10791
2927	99-19864-112	T	C	S	6861	10792
2928	99-19871-422	T	C	S	6862	10793
2929	99-19872-136	G	A	A	6863	10794
2930	99-19875-99	A	G	A	6864	10795
2931	99-19876-394	A	C	S	6865	10796
2932	99-19890-235	A	C	S	6866	10797
2933	99-19896-142	G	T	A	6867	10798
2934	99-19901-383	C	T	S	6868	10799
2935	99-19906-136	C	G	S	6869	10800
2936	99-19911-90	G	C	S	6870	10801
2937	99-19916-380	T	C	S	6871	10802
2938	99-19922-42	G	A	A	6872	10803
2939	99-19923-383	G	A	A	6873	10804
2940	99-19933-251	A	G	A	6874	10805
2941	99-19937-235	A	G	A	6875	10806
2942	99-19944-306	T	A	S	6876	10807
2943	99-19951-313	A	T	S	6877	10808
2944	99-20038-204	T	C	S	6878	10809
2945	99-20072-277	A	T	S	6879	10810
2946	99-20226-32	T	C	S	6880	10811
2947	99-20228-290	T	C	S	6881	10812
2948	99-20234-101	C	G	S	6882	10813
2949	99-20537-433	G	C	S	6883	10814
2950	99-20733-79	G	A	A	6884	10815
2951	99-20815-363	A	T	S	6885	10816
2952	99-20896-383	A	G	A	6886	10817
2953	99-20958-373	A	G	A	6887	10818
2954	99-21057-337	T	C	S	6888	10819
2955	99-21059-118	T	C	S	6889	10820
2956	99-21110-304	C	G	S	6890	10821
2957	99-21123-62	C	T	S	6891	10822
2958	99-21133-169	G	A	A	6892	10823
2959	99-21181-413	A	G	A	6893	10824
2960	99-21192-164	T	A	S	6894	10825
2961	99-21227-295	T	C	S	6895	10826

2962	99-21229-81	G	A	A	6896	10827
2963	99-21240-419	C	T	S	6897	10828
2964	99-21242-57	T	A	S	6898	10829
2965	99-21244-495	G	A	A	6899	10830
2966	99-21252-77	T	C	S	6900	10831
2967	99-21267-111	C	T	S	6901	10832
2968	99-21284-322	G	A	A	6902	10833
2969	99-21293-252	A	G	A	6903	10834
2970	99-21307-370	A	G	A	6904	10835
2971	99-21310-416	A	G	A	6905	10836
2972	99-21312-319	C	A	S	6906	10837
2973	99-21323-142	A	G	A	6907	10838
2974	99-21327-94	A	G	A	6908	10839
2975	99-21328-173	C	T	S	6909	10840
2976	99-21329-518	A	G	A	6910	10841
2977	99-21342-350	T	C	S	6911	10842
2978	99-21346-290	G	A	A	6912	10843
2979	99-21360-343	A	G	A	6913	10844
2980	99-21361-97	G	T	A	6914	10845
2981	99-21377-73	C	T	S	6915	10846
2982	99-21378-303	T	C	S	6916	10847
2983	99-21391-418	G	A	A	6917	10848
2984	99-21401-117	T	C	S	6918	10849
2985	99-21423-302	T	C	S	6919	10850
2986	99-21433-238	T	G	A	6920	10851
2987	99-21441-420	A	G	A	6921	10852
2988	99-21444-227	T	C	S	6922	10853
2989	99-21448-361	A	G	A	6923	10854
2990	99-21461-375	C	T	S	6924	10855
2991	99-21463-258	A	G	A	6925	10856
2992	99-21465-58	C	A	S	6926	10857
2993	99-21486-88	C	A	S	6927	10858
2994	99-21492-310	C	T	S	6928	10859
2995	99-21502-211	G	A	A	6929	10860
2996	99-21508-131	C	A	S	6930	10861
2997	99-21510-466	T	A	S	6931	10862
2998	99-21512-165	A	T	S	6932	10863
2999	99-21516-293	G	T	A	6933	10864
3000	99-21533-445	C	T	S	6934	10865
3001	99-21560-376	G	A	A	6935	10866
3002	99-21561-41	T	C	S	6936	10867
3003	99-21566-152	C	T	S	6937	10868
3004	99-21578-105	T	C	S	6938	10869
3005	99-21580-141	A	G	A	6939	10870
3006	99-21591-181	T	G	A	6940	10871
3007	99-21592-43	C	T	S	6941	10872
3008	99-21607-114	A	G	A	6942	10873
3009	99-21615-133	C	T	S	6943	10874
3010	99-21657-161	T	C	S	6944	10875
3011	99-21664-278	G	T	A	6945	10876
3012	99-21666-96	C	A	S	6946	10877
3013	99-21673-106	A	T	S	6947	10878
3014	99-21674-245	G	C	S	6948	10879
3015	99-21687-313	G	A	A	6949	10880
3016	99-21690-162	A	G	A	6950	10881
3017	99-21693-368	C	T	S	6951	10882

3018	99-21699-149	G	C	S	6952	10883
3019	99-21703-36	G	A	A	6953	10884
3020	99-21705-306	T	G	A	6954	10885
3021	99-21707-429	C	T	S	6955	10886
3022	99-21710-272	C	G	S	6956	10887
3023	99-21733-323	G	C	S	6957	10888
3024	99-21734-183	C	T	S	6958	10889
3025	99-21742-337	G	A	A	6959	10890
3026	99-21745-455	T	C	S	6960	10891
3027	99-21756-230	T	G	A	6961	10892
3028	99-21759-21	T	G	A	6962	10893
3029	99-21762-135	A	C	S	6963	10894
3030	99-21763-52	A	G	A	6964	10895
3031	99-21765-111	A	T	S	6965	10896
3032	99-21767-392	T	A	S	6966	10897
3033	99-21771-144	G	A	A	6967	10898
3034	99-21775-466	A	T	S	6968	10899
3035	99-21787-348	A	G	A	6969	10900
3036	99-21790-161	G	A	A	6970	10901
3037	99-21791-364	T	C	S	6971	10902
3038	99-21800-310	A	G	A	6972	10903
3039	99-21801-123	T	C	S	6973	10904
3040	99-21804-310	T	C	S	6974	10905
3041	99-21810-222	G	A	A	6975	10906
3042	99-21811-209	T	C	S	6976	10907
3043	99-21827-155	T	C	S	6977	10908
3044	99-21829-261	C	T	S	6978	10909
3045	99-21831-311	A	G	A	6979	10910
3046	99-21838-153	A	G	A	6980	10911
3047	99-21844-165	G	A	A	6981	10912
3048	99-21846-327	C	T	S	6982	10913
3049	99-21874-311	G	T	A	6983	10914
3050	99-21880-331	C	T	S	6984	10915
3051	99-21881-152	T	C	S	6985	10916
3052	99-21889-219	G	A	A	6986	10917
3053	99-21893-388	G	A	A	6987	10918
3054	99-21896-345	A	G	A	6988	10919
3055	99-21898-102	T	A	S	6989	10920
3056	99-21901-331	G	A	A	6990	10921
3057	99-21913-483	A	G	A	6991	10922
3058	99-21916-359	A	G	A	6992	10923
3059	99-21917-84	G	C	S	6993	10924
3060	99-21919-38	A	G	A	6994	10925
3061	99-21921-338	T	C	S	6995	10926
3062	99-21943-413	C	T	S	6996	10927
3063	99-21948-237	C	T	S	6997	10928
3064	99-21950-107	G	C	S	6998	10929
3065	99-21952-76	T	C	A	6999	10930
3066	99-21968-150	G	A	A	7000	10931
3067	99-21969-425	T	G	A	7001	10932
3068	99-22008-325	C	T	S	7002	10933
3069	99-22098-101	C	G	S	7003	10934
3070	99-22155-199	T	C	S	7004	10935
3071	99-22181-171	G	A	A	7005	10936
3072	99-22187-261	C	T	S	7006	10937
3073	99-22190-369	T	C	S	7007	10938

3074	99-22202-58	T	C	S	7008	10939
3075	99-22204-391	T	C	S	7009	10940
3076	99-22206-455	C	G	S	7010	10941
3077	99-22213-333	T	A	S	7011	10942
3078	99-22355-213	T	A	S	7012	10943
3079	99-22363-268	A	G	A	7013	10944
3080	99-22375-353	G	A	A	7014	10945
3081	99-22405-335	C	T	S	7015	10946
3082	99-2251-151	G	A	A	7016	10947
3083	99-22530-48	C	T	S	7017	10948
3084	99-22537-280	A	G	A	7018	10949
3085	99-22567-243	C	G	S	7019	10950
3086	99-22572-72	A	G	A	7020	10951
3087	99-22593-64	C	T	S	7021	10952
3088	99-22706-367	T	C	S	7022	10953
3089	99-22729-352	T	A	S	7023	10954
3090	99-22768-113	G	T	A	7024	10955
3091	99-22814-349	C	T	S	7025	10956
3092	99-22818-33	C	T	S	7026	10957
3093	99-22826-311	C	T	S	7027	10958
3094	99-22851-121	A	G	A	7028	10959
3095	99-23113-388	C	T	S	7029	10960
3096	99-23188-227	T	C	S	7030	10961
3097	99-23240-326	C	A	S	7031	10962
3098	99-23246-66	A	G	A	7032	10963
3099	99-23248-308	A	G	A	7033	10964
3100	99-23249-262	A	G	A	7034	10965
3101	99-23274-182	C	T	S	7035	10966
3102	99-2333-423	T	G	A	7036	10967
3103	99-2341-485	C	T	S	7037	10968
3104	99-2342-217	C	T	S	7038	10969
3105	99-23427-283	G	A	A	7039	10970
3106	99-23442-190	T	C	S	7040	10971
3107	99-23544-340	C	A	S	7041	10972
3108	99-23549-78	G	A	A	7042	10973
3109	99-23558-98	A	G	A	7043	10974
3110	99-23565-252	G	C	S	7044	10975
3111	99-23589-198	A	C	S	7045	10976
3112	99-23590-205	C	T	S	7046	10977
3113	99-23621-189	G	A	A	7047	10978
3114	99-23641-159	G	A	A	7048	10979
3115	99-23652-244	G	A	A	7049	10980
3116	99-23696-164	C	T	S	7050	10981
3117	99-23701-104	C	T	S	7051	10982
3118	99-23702-437	G	A	A	7052	10983
3119	99-2371-93	A	C	S	7053	10984
3120	99-23711-455	C	T	S	7054	10985
3121	99-23730-202	T	C	S	7055	10986
3122	99-23736-314	G	A	S	7056	10987
3123	99-23813-476	C	T	S	7057	10988
3124	99-23821-176	G	C	S	7058	10989
3125	99-23844-382	A	C	S	7059	10990
3126	99-23858-51	G	T	A	7060	10991
3127	99-23860-146	G	C	S	7061	10992
3128	99-23876-265	A	C	S	7062	10993
3129	99-23878-400	C	A	S	7063	10994

3130	99-23880-268	G	A	A	7064	10995
3131	99-23887-103	G	A	A	7065	10996
3132	99-23889-342	A	G	A	7066	10997
3133	99-23894-339	T	C	S	7067	10998
3134	99-23895-40	T	C	S	7068	10999
3135	99-23902-103	T	C	S	7069	11000
3136	99-23912-116	G	C	S	7070	11001
3137	99-23915-69	A	G	A	7071	11002
3138	99-23918-179	T	C	S	7072	11003
3139	99-23934-353	G	A	A	7073	11004
3140	99-23936-216	T	C	S	7074	11005
3141	99-23938-414	G	A	A	7075	11006
3142	99-23943-245	A	G	A	7076	11007
3143	99-23960-298	T	C	A	7077	11008
3144	99-23965-360	C	T	A	7078	11009
3145	99-23977-141	G	A	S	7079	11010
3146	99-23987-115	C	T	A	7080	11011
3147	99-23988-441	C	T	A	7081	11012
3148	99-23995-407	A	G	S	7082	11013
3149	99-24000-316	C	T	S	7083	11014
3150	99-24003-172	T	C	A	7084	11015
3151	99-24004-200	A	G	S	7085	11016
3152	99-24007-362	T	C	A	7086	11017
3153	99-24020-379	C	A	S	7087	11018
3154	99-24038-103	C	T	A	7088	11019
3155	99-24063-363	T	C	S	7089	11020
3156	99-24073-384	G	A	A	7090	11021
3157	99-24075-45	T	G	A	7091	11022
3158	99-24079-268	C	A	S	7092	11023
3159	99-24084-110	G	A	A	7093	11024
3160	99-24092-209	A	G	A	7094	11025
3161	99-24096-386	T	A	S	7095	11026
3162	99-24105-247	C	T	S	7096	11027
3163	99-24113-332	A	G	A	7097	11028
3164	99-24117-169	A	G	A	7098	11029
3165	99-24119-368	T	G	A	7099	11030
3166	99-24123-125	A	G	A	7100	11031
3167	99-24140-394	G	A	A	7101	11032
3168	99-24148-332	A	C	S	7102	11033
3169	99-24152-268	C	T	S	7103	11034
3170	99-24155-271	A	C	S	7104	11035
3171	99-24156-107	C	T	S	7105	11036
3172	99-24167-85	A	C	S	7106	11037
3173	99-24175-218	A	G	A	7107	11038
3174	99-24180-390	A	G	A	7108	11039
3175	99-24182-326	C	A	S	7109	11040
3176	99-24185-446	T	C	S	7110	11041
3177	99-24187-142	A	G	A	7111	11042
3178	99-24190-231	G	C	S	7112	11043
3179	99-24202-433	C	G	S	7113	11044
3180	99-24204-486	T	C	S	7114	11045
3181	99-24208-292	T	A	S	7115	11046
3182	99-24210-111	G	A	A	7116	11047
3183	99-24217-206	T	C	S	7117	11048
3184	99-24225-439	A	G	A	7118	11049
3185	99-24228-386	G	C	S	7119	11050

3186	99-24232-419	A	G	A	7120	11051
3187	99-24234-352	A	G	S	7121	11052
3188	99-24369-263	G	C	S	7122	11053
3189	99-24397-315	G	C	S	7123	11054
3190	99-24408-202	A	G	S	7124	11055
3191	99-2441-512	A	G	A	7125	11056
3192	99-24412-279	C	T	A	7126	11057
3193	99-24415-85	T	C	A	7127	11058
3194	99-24470-168	G	A	S	7128	11059
3195	99-24472-179	A	G	S	7129	11060
3196	99-24480-44	A	C	S	7130	11061
3197	99-24485-55	G	T	A	7131	11062
3198	99-24490-363	A	G	S	7132	11063
3199	99-24492-351	C	G	S	7133	11064
3200	99-24581-253	G	A	S	7134	11065
3201	99-24591-33	T	C	A	7135	11066
3202	99-24592-55	C	T	A	7136	11067
3203	99-24745-413	T	C	A	7137	11068
3204	99-24753-182	G	A	S	7138	11069
3205	99-24768-233	C	T	S	7139	11070
3206	99-24855-180	C	T	A	7140	11071
3207	99-24863-199	C	T	A	7141	11072
3208	99-24867-219	G	A	S	7142	11073
3209	99-24871-435	A	G	S	7143	11074
3210	99-24889-311	T	A	S	7144	11075
3211	99-24897-276	C	G	S	7145	11076
3212	99-24904-187	T	C	A	7146	11077
3213	99-24909-440	A	G	S	7147	11078
3214	99-24917-250	G	A	S	7148	11079
3215	99-24930-299	A	G	A	7149	11080
3216	99-24936-332	G	T	A	7150	11081
3217	99-24965-416	G	C	S	7151	11082
3218	99-24966-423	C	T	A	7152	11083
3219	99-25020-395	C	G	S	7153	11084
3220	99-25362-247	T	C	S	7154	11085
3221	99-25394-261	T	C	S	7155	11086
3222	99-25406-54	G	C	S	7156	11087
3223	99-25446-121	C	A	S	7157	11088
3224	99-25496-221	C	T	A	7158	11089
3225	99-25497-242	G	A	S	7159	11090
3226	99-2559-253	T	G	A	7160	11091
3227	99-25654-281	G	A	S	7161	11092
3228	99-2566-112	A	G	A	7162	11093
3229	99-2567-329	T	G	A	7163	11094
3230	99-2571-242	G	A	A	7164	11095
3231	99-25738-218	C	T	A	7165	11096
3232	99-25755-364	A	G	S	7166	11097
3233	99-25834-70	T	G	A	7167	11098
3234	99-25843-256	A	C	S	7168	11099
3235	99-26051-273	G	A	A	7169	11100
3236	99-26058-275	G	C	S	7170	11101
3237	99-26074-400	A	C	S	7171	11102
3238	99-26076-376	G	A	A	7172	11103
3239	99-2630-67	G	A	A	7173	11104
3240	99-2633-129	C	A	S	7174	11105
3241	99-2634-341	G	A	A	7175	11106

3242	99-2636-64	A	T	S	7176	11107
3243	99-2642-255	A	G	A	7177	11108
3244	99-2645-118	T	G	A	7178	11109
3245	99-2647-368	G	A	A	7179	11110
3246	99-2649-107	T	A	S	7180	11111
3247	99-2711-269	A	G	A	7181	11112
3248	99-2712-52	C	T	S	7182	11113
3249	99-2718-202	C	T	S	7183	11114
3250	99-2719-419	T	C	S	7184	11115
3251	99-2726-364	C	G	S	7185	11116
3252	99-2734-400	T	G	A	7186	11117
3253	99-2740-351	T	G	A	7187	11118
3254	99-2752-213	C	G	S	7188	11119
3255	99-2760-182	A	G	A	7189	11120
3256	99-2761-223	A	G	A	7190	11121
3257	99-2765-279	A	G	A	7191	11122
3258	99-2790-217	T	C	S	7192	11123
3259	99-2797-399	C	T	S	7193	11124
3260	99-2816-62	G	A	A	7194	11125
3261	99-2817-88	G	C	A	7195	11126
3262	99-2819-108	A	G	A	7196	11127
3263	99-2820-199	A	G	A	7197	11128
3264	99-2832-152	C	T	S	7198	11129
3265	99-2868-277	G	C	S	7199	11130
3266	99-2870-70	A	G	A	7200	11131
3267	99-2881-61	T	A	S	7201	11132
3268	99-2895-47	A	G	A	7202	11133
3269	99-2903-265	A	T	S	7203	11134
3270	99-2906-80	C	T	S	7204	11135
3271	99-2914-48	A	G	A	7205	11136
3272	99-2922-171	G	A	A	7206	11137
3273	99-2924-183	T	C	S	7207	11138
3274	99-2926-184	G	A	A	7208	11139
3275	99-2928-52	G	A	A	7209	11140
3276	99-2938-83	T	C	S	7210	11141
3277	99-2943-230	T	G	A	7211	11142
3278	99-2944-351	C	T	S	7212	11143
3279	99-295-355	T	C	S	7213	11144
3280	99-2954-160	C	G	S	7214	11145
3281	99-2956-239	C	T	S	7215	11146
3282	99-2970-318	G	C	S	7216	11147
3283	99-2978-135	C	A	S	7217	11148
3284	99-2981-53	T	C	S	7218	11149
3285	99-2988-243	C	T	S	7219	11150
3286	99-2989-345	C	A	S	7220	11151
3287	99-2991-256	G	A	A	7221	11152
3288	99-2995-168	C	T	S	7222	11153
3289	99-2999-371	C	T	S	7223	11154
3290	99-3013-250	A	T	S	7224	11155
3291	99-3018-50	A	G	A	7225	11156
3292	99-3019-316	A	T	S	7226	11157
3293	99-3020-369	A	G	A	7227	11158
3294	99-3021-290	A	G	A	7228	11159
3295	99-3044-216	C	T	S	7229	11160
3296	99-3045-108	C	T	S	7230	11161
3297	99-3046-91	T	C	S	7231	11162

3298	99-3047-395	G	A	A	7232	11163
3299	99-3058-420	T	A	S	7233	11164
3300	99-306-119	G	A	A	7234	11165
3301	99-3061-369	A	G	A	7235	11166
3302	99-3106-272	G	A	A	7236	11167
3303	99-3108-156	A	T	S	7237	11168
3304	99-3109-402	G	A	A	7238	11169
3305	99-3110-321	C	T	S	7239	11170
3306	99-312-311	C	T	S	7240	11171
3307	99-3129-113	T	A	S	7241	11172
3308	99-3132-158	A	G	A	7242	11173
3309	99-3144-112	A	G	A	7243	11174
3310	99-3147-24	C	G	S	7244	11175
3311	99-3153-190	C	T	S	7245	11176
3312	99-3154-110	T	C	S	7246	11177
3313	99-3156-251	T	C	S	7247	11178
3314	99-3167-227	A	G	A	7248	11179
3315	99-3195-71	G	A	A	7249	11180
3316	99-3217-274	G	A	A	7250	11181
3317	99-3224-232	A	G	A	7251	11182
3318	99-3231-109	T	A	S	7252	11183
3319	99-3234-274	A	C	S	7253	11184
3320	99-325-226	A	C	S	7254	11185
3321	99-3266-193	G	A	A	7255	11186
3322	99-3276-195	C	A	S	7256	11187
3323	99-3279-337	T	C	S	7257	11188
3324	99-3293-300	T	G	A	7258	11189
3325	99-3296-101	T	A	S	7259	11190
3326	99-3299-211	C	T	S	7260	11191
3327	99-3305-272	A	C	S	7261	11192
3328	99-3335-53	C	T	S	7262	11193
3329	99-3337-294	C	T	S	7263	11194
3330	99-3342-103	G	A	A	7264	11195
3331	99-3347-226	T	A	S	7265	11196
3332	99-3349-124	A	C	S	7266	11197
3333	99-3353-350	T	C	S	7267	11198
3334	99-3356-345	A	G	A	7268	11199
3335	99-3368-277	C	T	S	7269	11200
3336	99-3373-253	C	G	S	7270	11201
3337	99-3374-274	G	A	A	7271	11202
3338	99-3385-197	C	T	S	7272	11203
3339	99-3390-328	G	A	A	7273	11204
3340	99-3391-160	C	T	S	7274	11205
3341	99-3393-245	A	G	A	7275	11206
3342	99-3398-196	T	C	S	7276	11207
3343	99-3399-449	C	T	S	7277	11208
3344	99-3400-83	G	A	A	7278	11209
3345	99-3414-112	G	A	A	7279	11210
3346	99-3415-215	G	A	A	7280	11211
3347	99-3426-270	C	T	S	7281	11212
3348	99-3428-366	A	G	A	7282	11213
3349	99-3445-239	G	C	S	7283	11214
3350	99-3453-138	A	G	A	7284	11215
3351	99-3460-337	C	T	S	7285	11216
3352	99-3462-117	C	T	S	7286	11217
3353	99-3468-272	A	G	A	7287	11218

3354	99-3469-313	C	G	S	7288	11219
3355	99-3473-309	C	G	S	7289	11220
3356	99-3474-272	A	G	S	7290	11221
3357	99-3478-199	G	A	A	7291	11222
3358	99-3479-293	T	C	S	7292	11223
3359	99-3482-225	A	G	A	7293	11224
3360	99-3483-252	T	C	S	7294	11225
3361	99-3485-245	T	A	S	7295	11226
3362	99-3511-130	G	A	A	7296	11227
3363	99-3519-374	G	A	A	7297	11228
3364	99-3522-210	A	G	A	7298	11229
3365	99-3523-270	A	C	S	7299	11230
3366	99-3524-403	T	A	S	7300	11231
3367	99-3542-336	G	T	A	7301	11232
3368	99-3556-129	T	G	A	7302	11233
3369	99-3563-121	C	T	S	7303	11234
3370	99-3580-122	C	G	S	7304	11235
3371	99-3588-188	T	A	S	7305	11236
3372	99-3589-203	C	T	S	7306	11237
3373	99-3596-147	C	T	S	7307	11238
3374	99-36-69	C	T	S	7308	11239
3375	99-3601-226	T	C	S	7309	11240
3376	99-3603-80	T	A	S	7310	11241
3377	99-3604-91	A	G	A	7311	11242
3378	99-3619-330	C	T	S	7312	11243
3379	99-3620-314	G	A	A	7313	11244
3380	99-3628-31	G	A	A	7314	11245
3381	99-3629-219	G	A	A	7315	11246
3382	99-3631-159	C	T	S	7316	11247
3383	99-3638-259	A	C	S	7317	11248
3384	99-3641-230	C	A	S	7318	11249
3385	99-3666-280	G	A	A	7319	11250
3386	99-3667-190	G	A	A	7320	11251
3387	99-3677-196	T	A	S	7321	11252
3388	99-3680-274	C	G	S	7322	11253
3389	99-3689-50	A	G	A	7323	11254
3390	99-3690-355	G	C	S	7324	11255
3391	99-3699-230	G	A	A	7325	11256
3392	99-3702-226	T	A	S	7326	11257
3393	99-3703-331	C	T	S	7327	11258
3394	99-3705-195	G	A	A	7328	11259
3395	99-3709-366	T	C	S	7329	11260
3396	99-3717-68	A	G	A	7330	11261
3397	99-3728-341	T	C	S	7331	11262
3398	99-3739-215	G	A	A	7332	11263
3399	99-3746-337	C	G	S	7333	11264
3400	99-3749-174	C	T	S	7334	11265
3401	99-3752-210	C	T	S	7335	11266
3402	99-3760-59	A	G	A	7336	11267
3403	99-3761-329	C	T	S	7337	11268
3404	99-3764-198	C	T	S	7338	11269
3405	99-3765-279	A	G	A	7339	11270
3406	99-377-306	G	A	S	7340	11271
3407	99-3773-337	T	C	S	7341	11272
3408	99-3774-351	A	G	A	7342	11273
3409	99-3775-98	G	A	A	7343	11274

3410	99-3778-97	T	A	S	7344	11275
3411	99-3789-293	A	G	A	7345	11276
3412	99-3792-294	A	G	A	7346	11277
3413	99-3802-197	C	T	S	7347	11278
3414	99-3805-125	A	G	A	7348	11279
3415	99-3812-243	T	G	A	7349	11280
3416	99-3813-122	T	C	S	7350	11281
3417	99-3857-261	A	G	A	7351	11282
3418	99-3862-153	A	G	A	7352	11283
3419	99-3875-138	A	C	S	7353	11284
3420	99-3888-309	G	A	A	7354	11285
3421	99-3893-108	A	C	S	7355	11286
3422	99-3941-107	A	G	A	7356	11287
3423	99-3944-247	G	T	A	7357	11288
3424	99-3953-77	G	A	A	7358	11289
3425	99-3954-362	G	C	S	7359	11290
3426	99-3978-146	C	T	S	7360	11291
3427	99-3981-156	A	G	A	7361	11292
3428	99-3992-185	C	T	S	7362	11293
3429	99-4001-330	C	T	S	7363	11294
3430	99-4009-232	C	T	S	7364	11295
3431	99-4025-300	C	T	S	7365	11296
3432	99-4052-415	G	T	A	7366	11297
3433	99-4064-346	A	C	S	7367	11298
3434	99-4065-20	A	G	A	7368	11299
3435	99-4073-307	C	A	S	7369	11300
3436	99-4076-255	G	A	A	7370	11301
3437	99-4077-230	T	C	S	7371	11302
3438	99-4078-212	G	C	S	7372	11303
3439	99-4079-389	A	G	A	7373	11304
3440	99-4119-307	C	T	S	7374	11305
3441	99-4120-253	C	T	S	7375	11306
3442	99-4122-23	T	C	S	7376	11307
3443	99-4125-192	C	A	S	7377	11308
3444	99-4131-288	T	C	S	7378	11309
3445	99-4138-360	A	C	S	7379	11310
3446	99-4139-128	C	T	S	7380	11311
3447	99-4140-254	C	T	S	7381	11312
3448	99-4182-113	A	G	A	7382	11313
3449	99-4193-384	A	G	A	7383	11314
3450	99-4194-336	T	C	S	7384	11315
3451	99-4199-339	G	A	A	7385	11316
3452	99-4201-501	G	T	A	7386	11317
3453	99-4202-223	T	C	S	7387	11318
3454	99-4203-110	T	C	S	7388	11319
3455	99-4207-210	G	A	A	7389	11320
3456	99-4218-24	G	A	A	7390	11321
3457	99-4220-241	T	C	S	7391	11322
3458	99-4225-339	T	C	S	7392	11323
3459	99-4231-139	T	C	S	7393	11324
3460	99-4232-105	A	G	A	7394	11325
3461	99-4233-261	A	G	A	7395	11326
3462	99-4238-181	T	C	S	7396	11327
3463	99-4251-311	T	C	S	7397	11328
3464	99-4266-313	A	G	A	7398	11329
3465	99-4272-418	G	A	S	7399	11330

3466	99-4283-257	G	A	A	7400	11331
3467	99-4284-200	A	C	S	7401	11332
3468	99-4285-370	C	T	S	7402	11333
3469	99-4290-131	G	A	A	7403	11334
3470	99-4293-344	C	T	S	7404	11335
3471	99-4296-156	T	A	S	7405	11336
3472	99-4312-338	A	G	A	7406	11337
3473	99-4323-311	T	C	S	7407	11338
3474	99-4325-87	T	C	S	7408	11339
3475	99-4332-136	C	A	S	7409	11340
3476	99-4335-371	C	G	S	7410	11341
3477	99-4336-171	C	T	S	7411	11342
3478	99-4337-369	A	G	A	7412	11343
3479	99-4339-180	T	C	S	7413	11344
3480	99-4358-133	A	G	A	7414	11345
3481	99-4364-360	C	T	S	7415	11346
3482	99-4398-167	T	A	S	7416	11347
3483	99-4399-228	T	A	S	7417	11348
3484	99-4404-384	G	A	A	7418	11349
3485	99-4406-115	A	G	A	7419	11350
3486	99-4435-203	A	G	A	7420	11351
3487	99-4448-174	T	C	S	7421	11352
3488	99-4455-357	T	A	S	7422	11353
3489	99-4458-59	A	G	A	7423	11354
3490	99-4467-39	T	C	S	7424	11355
3491	99-4468-130	C	A	S	7425	11356
3492	99-4483-333	C	T	S	7426	11357
3493	99-4534-158	T	C	S	7427	11358
3494	99-4567-424	T	C	A	7428	11359
3495	99-4575-226	C	T	S	7429	11360
3496	99-4580-296	G	A	A	7430	11361
3497	99-4589-169	C	T	S	7431	11362
3498	99-4614-72	A	C	S	7432	11363
3499	99-4619-267	A	C	S	7433	11364
3500	99-4636-62	C	T	S	7434	11365
3501	99-4649-251	T	A	S	7435	11366
3502	99-468-271	T	C	S	7436	11367
3503	99-4688-442	C	G	S	7437	11368
3504	99-4691-400	A	G	A	7438	11369
3505	99-4692-372	T	G	A	7439	11370
3506	99-4715-280	G	A	A	7440	11371
3507	99-4736-164	C	T	S	7441	11372
3508	99-4744-72	G	T	A	7442	11373
3509	99-4746-160	G	T	A	7443	11374
3510	99-4748-76	C	T	S	7444	11375
3511	99-4755-84	C	A	S	7445	11376
3512	99-4758-66	G	A	A	7446	11377
3513	99-4772-80	C	A	S	7447	11378
3514	99-4791-198	G	A	S	7448	11379
3515	99-4792-298	G	A	A	7449	11380
3516	99-4799-209	C	A	S	7450	11381
3517	99-480-373	G	A	A	7451	11382
3518	99-4810-454	A	G	A	7452	11383
3519	99-4825-253	A	G	A	7453	11384
3520	99-4832-314	G	A	A	7454	11385
3521	99-4837-337	C	T	S	7455	11386

3522	99-4856-363	G	A	A	7456	11387
3523	99-4871-375	C	T	S	7457	11388
3524	99-4874-285	C	T	S	7458	11389
3525	99-4885-366	A	G	A	7459	11390
3526	99-49-41	G	A	A	7460	11391
3527	99-4903-395	A	G	A	7461	11392
3528	99-499-294	C	T	S	7462	11393
3529	99-5059-256	T	C	S	7463	11394
3530	99-5074-454	T	C	S	7464	11395
3531	99-5076-173	C	T	S	7465	11396
3532	99-5098-29	G	A	A	7466	11397
3533	99-51-263	G	C	S	7467	11398
3534	99-5112-188	C	T	S	7468	11399
3535	99-515-151	T	C	S	7469	11400
3536	99-5166-223	C	T	S	7470	11401
3537	99-5167-321	A	T	S	7471	11402
3538	99-5176-230	T	C	S	7472	11403
3539	99-5240-419	C	T	S	7473	11404
3540	99-5329-269	G	T	S	7474	11405
3541	99-5339-196	A	G	A	7475	11406
3542	99-5347-394	T	C	S	7476	11407
3543	99-5397-353	G	C	A	7477	11408
3544	99-55-233	C	A	S	7478	11409
3545	99-5549-289	A	G	A	7479	11410
3546	99-5569-237	A	G	A	7480	11411
3547	99-5575-330	C	T	S	7481	11412
3548	99-5602-372	G	C	S	7482	11413
3549	99-5671-333	T	C	S	7483	11414
3550	99-568-101	G	A	S	7484	11415
3551	99-5688-116	A	G	A	7485	11416
3552	99-5689-391	G	C	S	7486	11417
3553	99-5715-224	T	G	A	7487	11418
3554	99-5718-82	A	G	A	7488	11419
3555	99-5723-291	A	G	A	7489	11420
3556	99-5747-278	T	C	S	7490	11421
3557	99-5775-154	C	T	S	7491	11422
3558	99-5828-235	T	C	S	7492	11423
3559	99-5846-383	C	T	S	7493	11424
3560	99-5861-151	G	A	A	7494	11425
3561	99-59-137	A	C	S	7495	11426
3562	99-5930-449	T	A	S	7496	11427
3563	99-5931-330	G	A	A	7497	11428
3564	99-5967-165	C	T	S	7498	11429
3565	99-5987-135	A	G	A	7499	11430
3566	99-5996-279	T	C	S	7500	11431
3567	99-6001-372	T	G	A	7501	11432
3568	99-6020-477	A	G	A	7502	11433
3569	99-6047-225	T	A	S	7503	11434
3570	99-6071-272	A	G	A	7504	11435
3571	99-6076-394	A	T	S	7505	11436
3572	99-6096-354	C	G	S	7506	11437
3573	99-6103-356	T	C	S	7507	11438
3574	99-6124-125	T	C	S	7508	11439
3575	99-6173-229	T	G	A	7509	11440
3576	99-634-278	T	A	A	7510	11441
3577	99-6401-64	A	G	A	7511	11442

3578	99-6538-193	G	A	A	7512	11443
3579	99-6549-275	A	G	A	7513	11444
3580	99-6564-236	G	C	S	7514	11445
3581	99-6574-150	T	G	A	7515	11446
3582	99-6583-289	A	G	A	7516	11447
3583	99-6591-236	T	C	S	7517	11448
3584	99-6597-213	A	G	A	7518	11449
3585	99-6603-47	A	G	A	7519	11450
3586	99-6707-405	G	A	A	7520	11451
3587	99-6720-186	C	G	S	7521	11452
3588	99-6809-317	C	T	S	7522	11453
3589	99-6834-307	G	A	A	7523	11454
3590	99-6837-253	C	A	S	7524	11455
3591	99-6878-317	C	T	S	7525	11456
3592	99-6888-188	T	G	A	7526	11457
3593	99-6919-372	G	A	A	7527	11458
3594	99-6922-169	T	C	S	7528	11459
3595	99-6974-417	A	G	A	7529	11460
3596	99-6978-149	C	G	S	7530	11461
3597	99-6984-287	G	C	S	7531	11462
3598	99-6998-86	T	C	S	7532	11463
3599	99-7032-416	T	A	S	7533	11464
3600	99-7048-342	C	T	S	7534	11465
3601	99-7060-512	A	G	A	7535	11466
3602	99-7086-91	G	C	S	7536	11467
3603	99-7117-266	T	A	S	7537	11468
3604	99-7203-286	T	C	A	7538	11469
3605	99-7268-383	T	C	S	7539	11470
3606	99-7281-131	T	C	A	7540	11471
3607	99-7282-145	G	T	A	7541	11472
3608	99-7296-429	T	C	S	7542	11473
3609	99-7344-203	C	T	S	7543	11474
3610	99-7377-370	T	C	S	7544	11475
3611	99-7394-398	A	G	A	7545	11476
3612	99-7412-288	C	T	S	7546	11477
3613	99-7422-375	C	T	S	7547	11478
3614	99-7430-548	T	A	S	7548	11479
3615	99-7442-390	T	C	S	7549	11480
3616	99-7481-268	A	G	A	7550	11481
3617	99-7696-215	C	T	S	7551	11482
3618	99-7702-225	C	T	S	7552	11483
3619	99-7772-185	C	T	S	7553	11484
3620	99-7815-70	A	T	S	7554	11485
3621	99-7818-342	G	A	A	7555	11486
3622	99-7860-320	T	G	A	7556	11487
3623	99-7886-350	G	C	S	7557	11488
3624	99-7944-130	A	T	S	7558	11489
3625	99-7945-106	G	A	A	7559	11490
3626	99-7976-324	A	G	A	7560	11491
3627	99-8000-88	C	T	S	7561	11492
3628	99-8006-241	C	T	S	7562	11493
3629	99-8038-47	T	C	S	7563	11494
3630	99-8055-299	A	G	A	7564	11495
3631	99-8059-59	A	G	A	7565	11496
3632	99-8061-106	C	T	S	7566	11497
3633	99-8109-168	A	G	A	7567	11498

3634	99-8115-238	T	G	A	7568	11499
3635	99-8166-370	T	C	S	7569	11500
3636	99-8226-78	T	G	A	7570	11501
3637	99-8232-303	A	G	A	7571	11502
3638	99-824-359	C	T	S	7572	11503
3639	99-8274-70	A	G	A	7573	11504
3640	99-8359-153	T	A	S	7574	11505
3641	99-8630-298	G	A	A	7575	11506
3642	99-8659-399	T	A	S	7576	11507
3643	99-8679-371	T	G	A	7577	11508
3644	99-8690-117	T	C	S	7578	11509
3645	99-8751-299	C	T	S	7579	11510
3646	99-8795-58	G	A	A	7580	11511
3647	99-882-250	C	A	S	7581	11512
3648	99-887-344	G	T	A	7582	11513
3649	99-8875-283	G	A	A	7583	11514
3650	99-892-77	T	C	S	7584	11515
3651	99-8936-202	T	C	S	7585	11516
3652	99-8952-319	G	C	S	7586	11517
3653	99-896-83	T	C	S	7587	11518
3654	99-899-252	G	C	S	7588	11519
3655	99-9072-32	G	C	S	7589	11520
3656	99-9076-357	C	T	S	7590	11521
3657	99-9077-52	T	C	S	7591	11522
3658	99-9089-155	T	A	S	7592	11523
3659	99-9113-277	C	A	S	7593	11524
3660	99-9145-438	A	G	A	7594	11525
3661	99-9164-365	T	C	S	7595	11526
3662	99-9308-416	A	G	A	7596	11527
3663	99-9316-399	A	G	A	7597	11528
3664	99-9343-71	C	T	S	7598	11529
3665	99-9362-282	T	C	S	7599	11530
3666	99-9363-143	G	A	A	7600	11531
3667	99-9375-337	G	C	S	7601	11532
3668	99-9380-292	T	C	S	7602	11533
3669	99-9607-402	C	T	A	7603	11534
3670	99-9620-241	C	T	S	7604	11535
3671	99-9623-330	T	C	S	7605.	11536
3672	99-9633-32	T	C	S	7606	11537
3673	99-9636-423	T	C	S	7607	11538
3674	99-9658-42	T	G	A	7608	11539
3675	99-9662-213	T	C	A	7609	11540
3676	99-9666-363	T	C	A	7610	11541
3677	99-9668-185	C	T	A	7611	11542
3678	99-9680-363	A	G	S	7612	11543
3679	99-9696-292	T	C	A	7613	11544
3680	99-9697-375	A	C	S	7614	11545
3681	99-9700-289	C	T	A	7615	11546
3682	99-9704-445	T	C	A	7616	11547
3683	99-9706-448	C	T	A	7617	11548
3684	99-9709-115	T	C	A	7618	11549
3685	99-9710-242	C	A	S	7619	11550
3686	99-9714-302	T	C	S	7620	11551
3687	99-9717-449	A	G	A	7621	11552
3688	99-9726-190	T	C	S	7622	11553
3689	99-974-231	A	G	A	7623	11554

3690	99-9745-284	G	A	A	7624	11555
3691	99-9751-134	A	T	S	7625	11556
3692	99-9765-237	A	G	S	7626	11557
3693	99-9774-392	A	T	S	7627	11558
3694	99-9778-360	A	G	S	7628	11559
3695	99-9781-174	T	C	A	7629	11560
3696	99-9785-141	T	C	A	7630	11561
3697	99-9810-257	A	T	S	7631	11562
3698	99-9811-369	C	T	A	7632	11563
3699	99-9820-483	C	T	A	7633	11564
3700	99-9822-257	A	T	S	7634	11565
3701	99-9829-367	G	A	S	7635	11566
3702	99-983-278	G	A	A	7636	11567
3703	99-9832-128	T	C	A	7637	11568
3704	99-9833-167	A	G	S	7638	11569
3705	99-9835-217	C	T	A	7639	11570
3706	99-9837-275	A	G	S	7640	11571
3707	99-9839-416	G	C	S	7641	11572
3708	99-9840-192	C	T	A	7642	11573
3709	99-9847-25	A	G	S	7643	11574
3710	99-9849-291	G	A	S	7644	11575
3711	99-9852-276	T	C	A	7645	11576
3712	99-9854-316	G	C	S	7646	11577
3713	99-9856-252	C	T	A	7647	11578
3714	99-9859-132	C	A	S	7648	11579
3715	99-9866-365	T	C	A	7649	11580
3716	99-990-356	T	A	S	7650	11581
3717	99-9906-280	G	C	S	7651	11582
3718	99-9908-423	T	A	S	7652	11583
3719	99-991-157	A	G	A	7653	11584
3720	99-9915-281	T	G	A	7654	11585
3721	99-9920-245	T	C	A	7655	11586
3722	99-9921-365	T	C	A	7656	11587
3723	99-9922-154	C	T	A	7657	11588
3724	99-9926-454	G	A	S	7658	11589
3725	99-9928-454	C	T	A	7659	11590
3726	99-9929-144	G	A	S	7660	11591
3727	99-9935-418	G	A	S	7661	11592
3728	99-9941-426	T	A	S	7662	11593
3729	99-995-251	A	C	S	7663	11594
3730	99-996-210	T	C	S	7664	11595
3731	99-9986-202	T	C	S	7665	11596
3732	99-9988-111	T	G	A	7666	11597
3733	99-9994-226	C	A	S	7667	11598
3734	99-9995-50	C	T	S	7668	11599
3735	99-10069-366	A	T	S	7669	11600
3736	99-10074-266	A	G	A	7670	11601
3737	99-10129-177	A	G	S	7671	11602
3738	99-10198-271	A	G	S	7672	11603
3739	99-10306-345	C	T	A	7673	11604
3740	99-10307-115	A	G	S	7674	11605
3741	99-10326-149	C	T	A	7675	11606
3742	99-10393-179	A	G	S	7676	11607
3743	99-10685-454	A	C	S	7677	11608
3744	99-10857-217	C	T	A	7678	11609
3745	99-10948-281	C	T	A	7679	11610

3746	99-11104-329	A	G	S	7680	11611
3747	99-11116-199	C	T	A	7681	11612
3748	99-11117-282	A	G	S	7682	11613
3749	99-11121-461	A	G	S	7683	11614
3750	99-11124-363	C	T	A	7684	11615
3751	99-11172-373	C	T	A	7685	11616
3752	99-11206-379	C	T	A	7686	11617
3753	99-11303-223	C	T	A	7687	11618
3754	99-11307-168	G	T	A	7688	11619
3755	99-11325-188	A	G	S	7689	11620
3756	99-11365-273	C	T	A	7690	11621
3757	99-11389-268	A	T	S	7691	11622
3758	99-11395-376	A	G	S	7692	11623
3759	99-11500-50	C	T	S	7693	11624
3760	99-11571-88	G	T	S	7694	11625
3761	99-11710-452	A	G	S	7695	11626
3762	99-1173-208	A	T	S	7696	11627
3763	99-11735-215	C	T	A	7697	11628
3764	99-11864-218	A	C	S	7698	11629
3765	99-1187-293	G	C	S	7699	11630
3766	99-11872-228	C	T	A	7700	11631
3767	99-11878-212	C	T	A	7701	11632
3768	99-11905-202	G	C	S	7702	11633
3769	99-11932-48	C	T	A	7703	11634
3770	99-11964-158	A	C	S	7704	11635
3771	99-12164-412	C	T	A	7705	11636
3772	99-12227-278	G	C	S	7706	11637
3773	99-12417-447	A	G	S	7707	11638
3774	99-12459-119	G	T	A	7708	11639
3775	99-12521-212	C	T	S	7709	11640
3776	99-12569-95	A	G	A	7710	11641
3777	99-1298-430	A	G	S	7711	11642
3778	99-1315-105	G	C	S	7712	11643
3779	99-13154-74	C	T	A	7713	11644
3780	99-13155-134	A	G	S	7714	11645
3781	99-13249-461	C	T	A	7715	11646
3782	99-13794-147	G	C	S	7716	11647
3783	99-14899-215	A	C	S	7717	11648
3784	99-16351-44	C	T	A	7718	11649
3785	99-16436-382	A	G	S	7719	11650
3786	99-16753-387	G	C	S	7720	11651
3787	99-1807-300	A	G	A	7721	11652
3788	99-19032-132	A	C	S	7722	11653
3789	99-19212-369	C	T	S	7723	11654
3790	99-19273-219	A	G	A	7724	11655
3791	99-19279-356	C	T	S	7725	11656
3792	99-19541-172	A	G	A	7726	11657
3793	99-19552-214	G	T	A	7727	11658
3794	99-21051-435	C	T	S	7728	11659
3795	99-21246-20	C	T	S	7729	11660
3796	99-21387-465	C	T	S	7730	11661
3797	99-21407-352	A	G	A	7731	11662
3798	99-21418-83	C	T	S	7732	11663
3799	99-21419-85	C	T	S	7733	11664
3800	99-21430-308	C	T	S	7734	11665
3801	99-21435-96	A	G	A	7735	11666

3802	99-21446-240	C	T	S	7736	11667
3803	99-21452-173	A	G	A	7737	11668
3804	99-21488-376	G	T	A	7738	11669
3805	99-21489-227	C	T	S	7739	11670
3806	99-21496-248	C	T	S	7740	11671
3807	99-21519-446	A	G	A	7741	11672
3808	99-21618-178	A	G	A	7742	11673
3809	99-21725-371	C	T	S	7743	11674
3810	99-21773-155	A	C	S	7744	11675
3811	99-21781-252	A	G	A	7745	11676
3812	99-21820-230	A	G	A	7746	11677
3813	99-21822-50	A	G	A	7747	11678
3814	99-21939-170	A	T	S	7748	11679
3815	99-22404-59	A	G	A	7749	11680
3816	99-22594-395	A	G	A	7750	11681
3817	99-22679-148	A	C	S	7751	11682
3818	99-23095-184	G	C	S	7752	11683
3819	99-23370-249	C	T	S	7753	11684
3820	99-23568-395	G	C	S	7754	11685
3821	99-23824-339	C	T	S	7755	11686
3822	99-23969-316	C	T	A	7756	11687
3823	99-24032-138	A	T	S	7757	11688
3824	99-24048-286	C	T	S	7758	11689
3825	99-24074-190	A	G	A	7759	11690
3826	99-24082-408	A	C	S	7760	11691
3827	99-24104-308	G	T	A	7761	11692
3828	99-24138-224	A	G	A	7762	11693
3829	99-24172-116	C	T	S	7763	11694
3830	99-24267-190	A	C	S	7764	11695
3831	99-24949-289	C	T	A	7765	11696
3832	99-253-97	A	G	A	7766	11697
3833	99-2694-411	A	G	A	7767	11698
3834	99-2697-336	A	G	A	7768	11699
3835	99-2720-280	A	G	A	7769	11700
3836	99-2851-105	G	C	S	7770	11701
3837	99-2889-197	C	T	S	7771	11702
3838	99-3072-323	A	G	A	7772	11703
3839	99-3089-49	A	G	A	7773	11704
3840	99-3157-203	G	T	A	7774	11705
3841	99-3210-341	G	T	A	7775	11706
3842	99-3218-344	A	G	A	7776	11707
3843	99-3251-254	G	T	A	7777	11708
3844	99-3298-158	C	T	A	7778	11709
3845	99-3300-433	A	G	A	7779	11710
3846	99-3364-247	A	T	S	7780	11711
3847	99-3427-271	A	G	A	7781	11712
3848	99-3484-96	A	G	A	7782	11713
3849	99-3537-196	A	G	A	7783	11714
3850	99-3568-156	G	T	A	7784	11715
3851	99-3592-325	A	G	A	7785	11716
3852	99-3602-245	C	T	S	7786	11717
3853	99-3608-264	A	G	A	7787	11718
3854	99-3643-352	A	G	A	7788	11719
3855	99-3770-363	C	T	S	7789	11720
3856	99-3772-266	A	G	A	7790	11721
3857	99-3790-361	A	G	A	7791	11722

3858	99-3818-255	A	G	A	7792	11723
3859	99-3863-328	A	G	A	7793	11724
3860	99-3879-245	A	G	A	7794	11725
3861	99-3882-312	C	T	S	7795	11726
3862	99-3883-329	C	T	S	7796	11727
3863	99-3884-355	G	C	S	7797	11728
3864	99-3894-333	C	T	S	7798	11729
3865	99-3936-352	A	G	S	7799	11730
3866	99-3946-236	A	G	A	7800	11731
3867	99-4029-174	C	T	S	7801	11732
3868	99-4036-308	C	T	S	7802	11733
3869	99-4102-109	A	G	A	7803	11734
3870	99-4110-180	A	G	A	7804	11735
3871	99-4111-259	A	G	A	7805	11736
3872	99-4126-366	A	G	A	7806	11737
3873	99-4157-72	A	G	A	7807	11738
3874	99-4228-168	C	T	S	7808	11739
3875	99-4239-328	A	G	A	7809	11740
3876	99-4254-307	A	G	A	7810	11741
3877	99-4264-228	C	T	S	7811	11742
3878	99-4311-146	A	G	A	7812	11743
3879	99-4381-385	C	T	S	7813	11744
3880	99-4403-194	A	C	S	7814	11745
3881	99-4524-296	A	G	A	7815	11746
3882	99-4582-359	G	T	A	7816	11747
3883	99-4611-151	C	T	S	7817	11748
3884	99-4689-375	A	T	S	7818	11749
3885	99-4762-114	A	G	A	7819	11750
3886	99-4878-107	C	T	S	7820	11751
3887	99-5075-219	C	T	A	7821	11752
3888	99-5190-277	A	G	A	7822	11753
3889	99-5605-90	G	T	A	7823	11754
3890	99-5882-105	C	T	S	7824	11755
3891	99-5977-241	C	T	S	7825	11756
3892	99-5993-323	C	T	S	7826	11757
3893	99-5994-205	G	T	S	7827	11758
3894	99-6827-399	A	G	A	7828	11759
3895	99-7076-198	C	T	S	7829	11760
3896	99-7215-279	C	T	S	7830	11761
3897	99-8206-133	A	G	A	7831	11762
3898	99-8614-236	A	G	A	7832	11763
3899	99-889-153	G	C	S	7833	11764
3900	99-9450-70	A	T	S	7834	11765
3901	99-9609-220	C	T	A	7835	11766
3902	99-9612-324	A	G	S	7836	11767
3903	99-9616-136	A	G	A	7837	11768
3904	99-9683-49	A	G	S	7838	11769
3905	99-9907-88	C	T	A	7839	11770
3906	99-993-218	C	T	S	7840	11771
3907	99-24069-351	C	T	S	7841	11772
3908	99-3855-279	G	C	A	7842	11773
3909	99-344-439	G	A	A	7843	11774
3910	99-366-274	C	T	S	7844	11775
3911	99-359-308	A	G	A	7845	11776
3912	99-355-219	A	G	A	7846	11777
3913	99-365-344	C	T	S	7847	11778

3914	99-2452-54	C	T	S	7848	11779
3915	99-123-381	C	T	S	7849	11780
3916	4-26-29	A	G	A	7850	11781
3917	4-14-240	C	T	S	7851	11782
3918	4-77-151	G	C	S	7852	11783
3919	99-217-277	C	T	S	7853	11784
3920	4-67-40	C	T	S	7854	11785
3921	99-213-164	A	G	A	7855	11786
3922	99-221-377	A	C	S	7856	11787
3923	99-135-196	A	G	A	7857	11788
3924	99-1482-32	A	C	S	7858	11789
3925	4-73-134	G	C	S	7859	11790
3926	4-65-324	C	T	S	7860	11791
3927	10-32-357	A	C	S	7861	11792
3928	10-33-175	T	C	S	7862	11793
3929	10-33-234	A	C	S	7862	11793
3930	10-33-327	C	T	S	7862	11793
3931	10-35-358	G	C	A	7863	11794
3932	10-35-390	T	C	S	7863	11794
3933	10-36-164	A	G	A	7864	11795
3934	10-204-326	A	G	A	7865	11796

Sequence listing free text

The following free text appears in the accompanying Sequence Listing:

upstream

downstream

amplification

primer

polymorphic

base

in

for

SEQ

complement

sequence

WHAT IS CLAIMED IS:

1. An isolated or purified polynucleotide comprising a contiguous span of at least 12 nucleotides of a sequence selected from the group consisting of SEQ ID No. 1 to 2260, and the complements thereof.

2. A polynucleotide according to claim 1, wherein said span comprises a map-related biallelic marker.

3. An isolated or purified polynucleotide consisting essentially of a contiguous span of at least 8 to 43 nucleotides of a sequence selected from the group consisting of SEQ ID No. 2261 to 3734, 3735 to 3908, and the complements thereof.

4. A polynucleotide according to claim 3, wherein said span comprises a map-related biallelic marker.

5. An isolated or purified polynucleotide comprising a contiguous span of at least 12 nucleotides of a sequence selected from the group consisting of SEQ ID No. 2261 to 3734, and the complements thereof, wherein said span comprises a map-related biallelic marker and the 1st allele indicated in Table 1 is present at said map-related biallelic marker.

6. A polynucleotide according to any one of claims 2, 4, and 5, wherein said contiguous span is 18 to 35 nucleotides in length and said biallelic marker is within 4 nucleotides of the center of said polynucleotide.

7. A polynucleotide according to claim 6, wherein said polynucleotide consists essentially of said contiguous span and said contiguous span is 25 nucleotides in length and said biallelic marker is at the center of said polynucleotide.

8. An isolated or purified polynucleotide comprising a contiguous span of at least 12 nucleotides of a sequence selected from the group consisting of SEQ ID No. 3935 to 6194, 7866 to 10125, and the complements thereof.

9. An isolated or purified polynucleotide consisting essentially of a contiguous span of at least 8 to 43 nucleotides of a sequence selected from the group consisting of SEQ ID No. 6195 to 7668, 7669 to 7842, 10126 to 11599, 11600 to 11773, and the complements thereof.

10. A polynucleotide according to any one of claims 1, 3, 8, and 9, wherein the 3' end of said contiguous span is present at the 3' end of said polynucleotide.

11. A polynucleotide according to any one of claims 2, 3, and 5, wherein the 3' end of said contiguous span is located at the 3' end of said polynucleotide and said biallelic marker is present at the 3' end of said polynucleotide.

12. A polynucleotide according to either of claims 1 and 3, wherein the 3' end of said contiguous span is present at the 3' end of said polynucleotide and the 3' end of said polynucleotide is located within 10 nucleotides upstream of a map-related biallelic marker in said sequence.

13. A polynucleotide according to claim 12, wherein the 3' end of said polynucleotide is located 1 nucleotide upstream of a map-related biallelic marker in said sequence.

14. A polynucleotide according to claim 13, wherein said contiguous span is 19 nucleotides in length and said polynucleotide consists of said contiguous span.

15. A polynucleotide according to any one of claims 1, 3, 5, 8, and 9 wherein said contiguous span comprises at least 21 contiguous nucleotides.

16. A polynucleotide according to any one of claims 1, 3, and 5, wherein said contiguous span comprises at least 30 contiguous nucleotides.

17. A polynucleotide according to any one of claims 1, 3, and 5, wherein said contiguous span comprises at least 43 contiguous nucleotides.

18. A polynucleotide for use in determining the identity of nucleotides at a map-related biallelic marker, wherein said determining is performed in a hybridization assay, sequencing assay, microsequencing assay, or an enzyme-based mismatch detection assay.

19. A polynucleotide for use in amplifying a segment of nucleotides comprising a map-related biallelic marker.

20. A polynucleotide according to either of claims 18 and 19, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, and the complements thereto.

21. A polynucleotide according to either of claims 18 and 19, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 2260, 2261 to 3734, and the complements thereto.

22. A polynucleotide according to any one of claims 1, 3, 5, 8, 9, 18, and 19 attached to a solid support.

23. An array of polynucleotides comprising at least one polynucleotide according to claim 22.

24. An array according to claim 23, wherein said array is addressable.

25. A polynucleotide according to any one of claims 1, 3, 5, 7, 8, 9, 14, 18, and 19, further comprising a label.

26. A map of the human genome comprising an ordered array of biallelic markers, wherein at least 1 of said biallelic markers is a map-related biallelic marker.

27. A map of according to claim 26, comprising all of the biallelic markers of SEQ ID Nos. 1 to 3908, and the complements thereto.

28. A method of genotyping comprising determining the identity of a nucleotide at a map-related biallelic marker in a biological sample.

29. A method according to claim 28, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, and the complements thereto.

30. A method according to claim 28, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 2260, 2261 to 3734, and the complements thereto.

31. A method according to claim 28, wherein said biological sample is derived from a single subject.

32. A method according to claim 31, wherein the identity of the nucleotides at said biallelic marker is determined for both copies of said biallelic marker present in said subject's genome.

33. A method according claim 28, wherein said biological sample is derived from multiple subjects.

34. A method according to claim 28, further comprising amplifying a portion of said sequence comprising the biallelic marker prior to said determining step.

35. A method according to claim 34, wherein said amplifying is performed by PCR.

36. A method according to claim 28, wherein said determining is performed by a hybridization assay, a sequencing assay, a microsequencing assay, or an enzyme-based mismatch detection assay.

37. A method of determining the frequency in a population of an allele of a map-related biallelic marker, comprising:

a) genotyping individuals from said population for said biallelic marker according to the method of claim 28; and

b) determining the proportional representation of said biallelic marker in said population.

38. A method according to claim 37, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, and the complements thereto.

39. A method according to claim 37, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 2260, 2261 to 3734, and the complements thereto.

40. A method according to claim 37, wherein said genotyping of step a) is performed on each individual of said population.

41. A method according to claim 37, wherein said genotyping is performed on a single biological sample derived from said population.

42. A method of detecting an association between an allele and a phenotype, comprising the steps of:

a) determining the frequency of at least one map-related biallelic marker allele in a trait positive population according to the method of claim 37;

b) determining the frequency of said map-related biallelic marker allele in a control population according to the method of claim 37; and

c) determining whether a statistically significant association exists between said allele and said phenotype.

43. A method of estimating the frequency of a haplotype for a set of biallelic markers in a population, comprising:

a) genotyping each individual in said population for at least one map-related biallelic marker according to claim 31;

b) genotyping each individual in said population for a second biallelic marker by determining the identity of the nucleotides at said second biallelic marker for both copies of said second biallelic marker present in the genome; and

c) applying a haplotype determination method to the identities of the nucleotides determined in steps a) and b) to obtain an estimate of said frequency.

44. A method according to claim 43, wherein said haplotype determination method is selected from the group consisting of asymmetric PCR amplification, double PCR amplification of specific alleles, the Clark method, or an expectation maximization algorithm.

45. A method according to claim 43, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908, and the complements thereto.

46. A method according to claim 43, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 2260, 2261 to 3734, and the complements thereto.

47. A method of detecting an association between a haplotype and a phenotype, comprising the steps of:

a) estimating the frequency of at least one haplotype in a trait positive population according to the method of claim 43;

b) estimating the frequency of said haplotype in a control population according to the method of claim 43; and

c) determining whether a statistically significant association exists between said haplotype and said phenotype.

48. A method according to either claim 42 or 47, wherein said control population is a trait negative population.

49. A method according to either claim 42 or 47, wherein said case control population is a random population.

50. A method according to claim 42, wherein each of said genotyping of steps a) and b) is performed on a single pooled biological sample derived from each of said populations.

51. A method according to claim 42, wherein said genotyping of steps a) and b) is performed separately on biological samples derived from each individual in said populations.

52. A method according to either claim 42 or 47, wherein said phenotype is selected from the group consisting of disease, drug response, drug efficacy, treatment response, treatment efficacy, and drug toxicity.

53. A method according to claim 42, wherein the identity of the nucleotides at all of the biallelic markers of SEQ ID Nos. 1 to 3908 is determined in steps a) and b).

54. A computer readable medium having stored thereon the sequence of the polynucleotide according to any one of the claims selected from the group consisting of 1, 3, 5, 7, 8, 9 and 14.

55. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon the sequence of the polynucleotide according to any one of the claims selected from the group consisting of 1, 3, 5, 7, 8, 9 and 14.

56. The computer system of Claim 55, further comprising a sequence comparer and a data storage device having reference sequences stored thereon.

57. A method for comparing a first sequence to a reference sequence, comprising the steps of:

a) reading said first sequence and said reference sequence through use of a computer program which compares sequences; and

b) determining differences between said first sequence and said reference sequence with said computer program;

wherein said first sequence is the sequence of the polynucleotide according to any one of the claims selected from the group consisting of 1, 3, 5, 7, 8, 9 and 14.

58. A diagnostic kit comprising a polynucleotide according to any one of claims 1, 3, 5, 7, 8, 9, 14, 18, and 19.

59. A method of identifying a gene associated with a detectable trait comprising the steps of:

a) determining the frequency of each allele of at least one map-related biallelic marker in individuals having said detectable trait and individuals lacking said detectable trait according to the method of claim 41;

b) identifying at least one allele of said biallelic marker having a statistically significant association with said detectable trait; and

c) identifying a gene in linkage disequilibrium with said allele.

60. The method according to claim 59, further comprising the step of: d) identifying a mutation in gene which is associated with said detectable trait.

61. A method of identifying biallelic markers associated with a detectable trait comprising the steps of:

a) determining the frequencies of a set of biallelic markers comprising at least one map-related biallelic marker in individuals who express said detectable trait and individuals who do not express said detectable trait; and

b) identifying at least one biallelic marker in said set which are statistically associated with the expression of said detectable trait.

62. A method for determining whether an individual is at risk of developing a detectable trait or suffers from a detectable trait associated with said trait comprising the steps of:

a) obtaining a nucleic acid sample from said individual;

b) screening said nucleic acid sample with at least one map-related biallelic marker; and

c) determining whether said nucleic acid sample contains at least one biallelic marker statistically associated with said detectable trait.

63. The method according to any one of claims 59, 61 and 62, wherein said detectable trait is selected from the group consisting of disease, drug response, drug efficacy, treatment response, treatment efficacy, and drug toxicity.

64. A method of administering a drug or treatment comprising:

a) obtaining a nucleic acid sample from an individual;

b) determining the identity of the polymorphic base of at least one map-related biallelic marker according to the method of claim 31 which is associated with a positive response to said drug or treatment, or at least one map-related biallelic marker which is associated with a negative response to said drug or treatment; and

c) administering said drug or treatment to said individual if said nucleic acid sample contains at least one biallelic marker associated with a positive response to said drug or treatment, or if said nucleic acid sample lacks at least one biallelic markers associated with a negative response to said drug or treatment.

65. A method of selecting an individual for inclusion in a clinical trial of a drug or treatment comprising:

a) obtaining a nucleic acid sample from an individual;

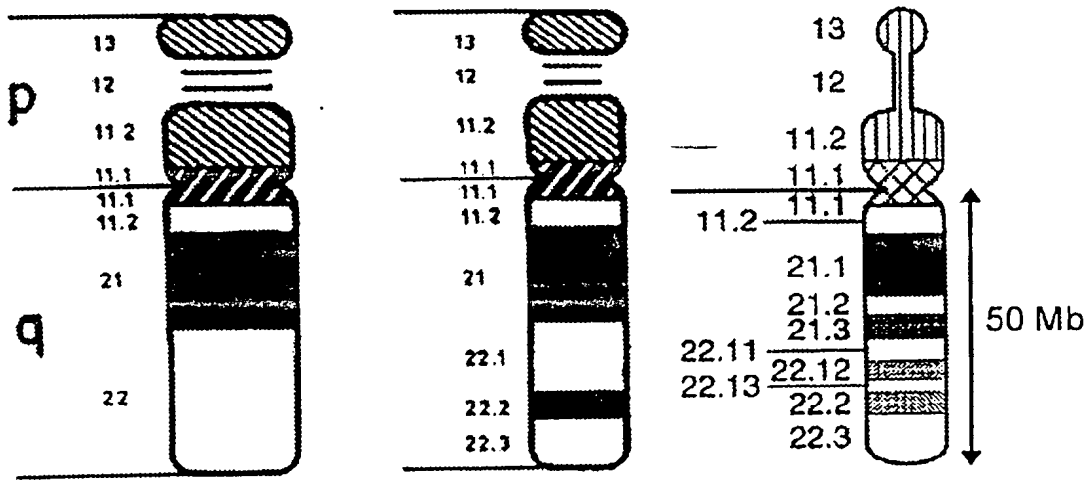
b) determining the identity of the polymorphic base of at least one map-related biallelic marker according to the method of claim 31 which is associated with a positive response to said drug or treatment, or at least one biallelic marker associated with a negative response to said drug or treatment in said nucleic acid sample; and

c) including said individual in said clinical trial if said nucleic acid sample contains at least one biallelic marker which is associated with a positive response to said drug or treatment, or if said nucleic acid sample lacks at least one biallelic markers associated with a negative response to said drug or treatment.

66. The method according to either of claims 64 and 65, wherein said administering step comprises administering said drug or treatment to said individual if said nucleic acid sample contains at least one biallelic marker associated with a positive response to said drug or treatment, and said nucleic acid sample lacks at least one biallelic marker associated with a negative response to said drug or treatment.

67. The method according to any one of claims 59, 61, 62, 64, and 65, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3908.

68. The method according to any one of claims 59, 61, 62, 64, and 65, wherein said map-related biallelic marker is selected from the group consisting of the biallelic markers of SEQ ID Nos. 1 to 3734.


p
q
13
12
11.2
11.1
11.1
11.2
21
22
22.1
22.2
22.3
11.2
21.1
21.2
21.3
22.11
22.12
22.13
50 Mb

21

Figure 1

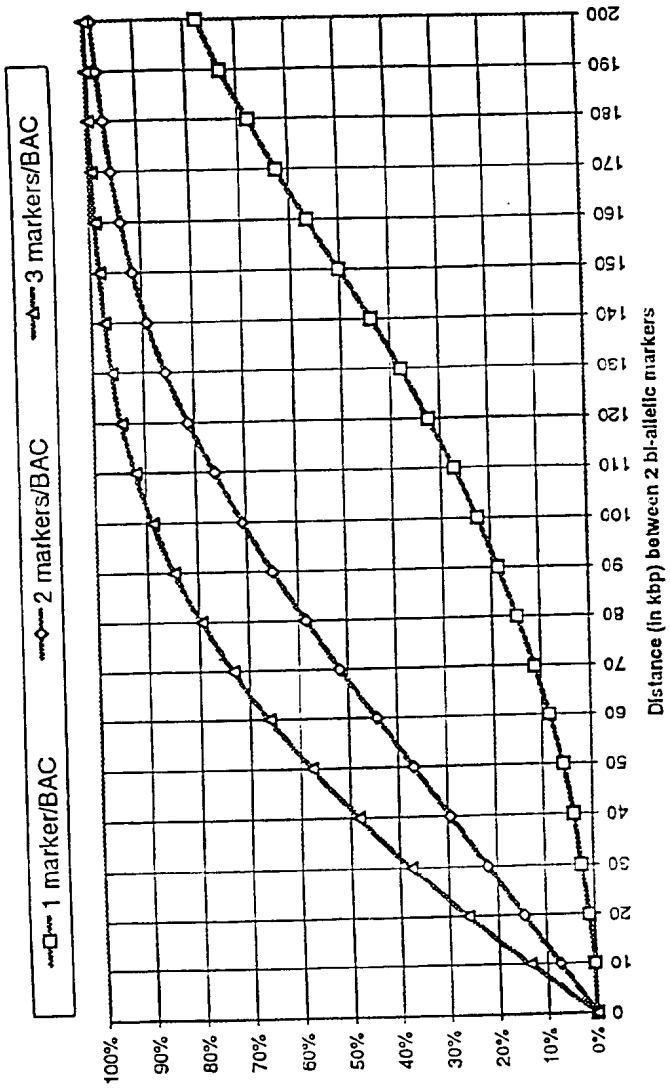

1 marker/BAC
2 markers/BAC
3 markers/BAC
Distance (in kbp) between 2 bi-allelic markers
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

Figure 2A

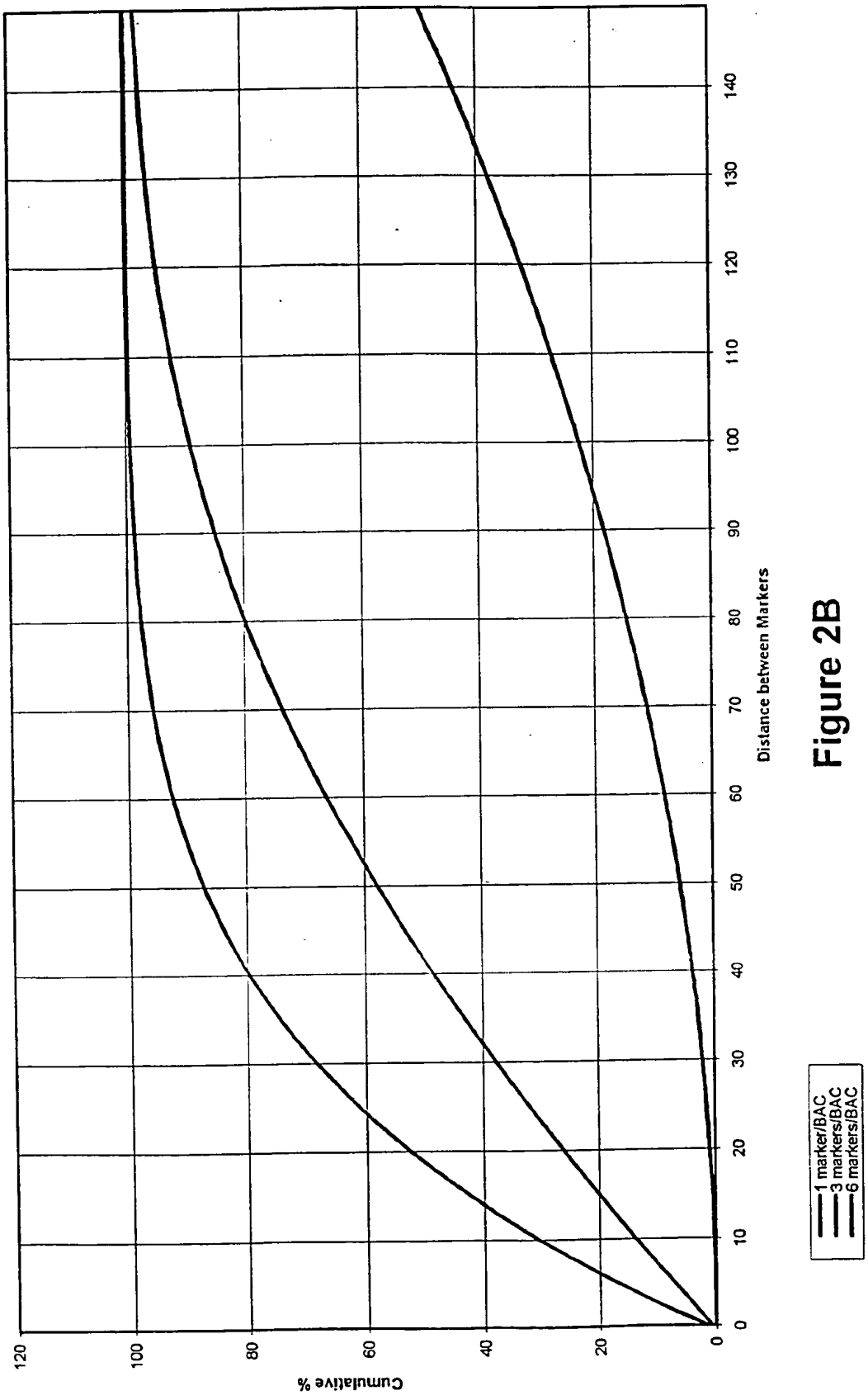

Cumulative %
120
100
80
60
40
20
0
Distance between Markers
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
1 marker/BAC
3 markers/BAC
6 markers/BAC

Figure 2B

p-VALUE DISTRIBUTION

# aff	150						
# non aff	150						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	8,7699E-05	0,06407752	0,14252002	0,19106311	0,21543442	0,22009395
Δ pAi	0,1	1,9149E-08	0,00060364	0,00467774	0,01023571	0,01382303	0,01382303
Δ pAi	0,15	3,0618E-12	1,3319E-06	3,8827E-05	0,0001478	0,0002343	0,00020218
Δ pAi	0,2	3,2153E-16	9,1413E-10	9,0305E-08	5,733E-07	9,6336E-07	5,733E-07
Δ pAi	0,25	2,0791E-20	2,2614E-13	6,2679E-11	5,873E-10	8,7113E-10	2,5396E-10
Δ pAi	0,3	7,8245E-25	2,152E-17	1,3261E-14	1,5189E-13	1,5189E-13	1,3261E-14
Δ pAi	0,35	1,6192E-29	7,9823E-22	8,4152E-19	9,1669E-18	4,2713E-18	5,5844E-20
Δ pAi	0,4	1,7336E-34	1,1282E-26	1,524E-23	1,1488E-22	1,524E-23	1,1282E-26

# aff	200						
# non aff	200						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	5,9233E-06	0,03250945	0,09039173	0,13111935	0,15260313	0,15678006
Δ pAi	0,1	8,649E-11	7,4765E-05	0,00109084	0,00302686	0,00447365	0,00447365
Δ pAi	0,15	8,0215E-16	2,3653E-08	2,0257E-06	1,1771E-05	2,1573E-05	1,7772E-05
Δ pAi	0,2	4,1762E-21	1,5375E-12	6,7374E-10	7,764E-09	1,5417E-08	7,764E-09
Δ pAi	0,25	1,1282E-26	2,525E-17	4,4025E-14	8,5532E-13	1,4423E-12	2,8149E-13
Δ pAi	0,3	1,4722E-32	1,1488E-22	5,8424E-19	1,4886E-17	1,4886E-17	5,8424E-19
Δ pAi	0,35	8,6169E-39	1,4784E-28	1,5457E-24	3,6958E-23	1,3394E-23	4,197E-26
Δ pAi	0,4	2,0885E-45	5,2308E-35	7,6438E-31	1,1224E-29	7,6438E-31	5,2308E-35

# aff	affected individuals
# non aff	non affected individuals
pAi non aff	allele frequency in non affected individuals
Δ pAi	% Difference in allele frequency between affected and non-affected individuals

Figure 3 (I)

p-VALUE DISTRIBUTION

# aff	500						
# non aff	500						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	8,0004E-13	0,00072323	0,00741965	0,0169842	0,02371865	0,02516449
Δ pAi	0,1	1,0695E-24	3,7948E-10	2,4176E-07	2,7579E-06	6,9679E-06	6,9679E-06
Δ pAi	0,15	3,813E-37	1,0719E-18	5,8344E-14	4,2622E-12	1,8601E-11	1,1611E-11
Δ pAi	0,2	2,9626E-50	5,0895E-29	1,6881E-22	6,9321E-20	3,7441E-19	6,9321E-20
Δ pAi	0,25	4,2697E-64	7,2043E-41	7,7528E-33	1,194E-29	4,3462E-29	7,6438E-31
Δ pAi	0,3	9,6976E-79	3,9328E-54	6,3017E-45	1,9429E-41	1,9429E-41	6,3017E-45
Δ pAi	0,35	2,911E-94	8,8513E-69	8,7879E-59	2,3478E-55	1,8839E-56	1,1206E-62
Δ pAi	0,4	9,505E-111	7,7199E-85	1,8063E-74	1,4484E-71	1,8063E-74	7,7199E-85

# aff	150						
# non aff	850						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	2,1561E-20	0,00994614	0,04896055	0,08358651	0,10417953	0,11025423
Δ pAi	0,1	2,0126E-39	5,571E-07	0,00010149	0,00058665	0,00119145	0,00139743
Δ pAi	0,15	1,1091E-58	2,7555E-13	8,462E-09	2,9851E-07	1,2395E-06	1,6229E-06
Δ pAi	0,2	3,2726E-78	2,1683E-21	3,2211E-14	1,1049E-11	1,111E-10	1,5638E-10
Δ pAi	0,25	4,9576E-98	4,4952E-31	6,5226E-21	3,1015E-17	2,5169E-16	1,1763E-15
Δ pAi	0,3	3,749E-118	3,6987E-42	8,129E-29	6,9335E-24	5,4331E-22	6,5657E-22
Δ pAi	0,35	1,383E-138	1,6797E-54	7,1058E-38	1,2938E-31	2,9415E-29	2,5869E-29
Δ pAi	0,4	2,435E-159	5,4915E-68	4,8846E-48	2,1003E-40	1,3332E-37	6,8178E-38

# aff	affected individuals
# non aff	non affected individuals
pAi non aff	allele frequency in non affected individuals
Δ pAi	% Difference in allele frequency between affected and non-affected individuals

Figure 3 (II)

p-VALUE DISTRIBUTION

# aff	200						
# non aff	500						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	1,0628E-12	0,00789803	0,03942584	0,06867566	0,08621572	0,09083704
Δ pAi	0,1	3,4525E-24	4,4217E-07	5,6883E-05	0,00031976	0,0006363	0,00070881
Δ pAi	0,15	5,9036E-36	4,3025E-13	3,3635E-09	9,2134E-08	3,319E-07	3,5871E-07
Δ pAi	0,2	4,7325E-48	1,5566E-20	1,0346E-14	1,7218E-12	1,1512E-11	1,0047E-11
Δ pAi	0,25	1,6694E-60	3,5436E-29	2,0473E-21	2,2178E-18	1,1498E-17	1,3524E-17
Δ pAi	0,3	2,4613E-73	7,2498E-39	3,0748E-29	2,0601E-25	3,4525E-24	7,4807E-25
Δ pAi	0,35	1,4447E-86	1,6945E-49	3,9559E-38	1,4118E-33	2,662E-32	1,4118E-33
Δ pAi	0,4	3,214E-100	5,3051E-61	4,7325E-48	7,1282E-43	1,0691E-41	7,2652E-44

# aff	500						
# non aff	1000						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	6,4766E-24	5,7827E-05	0,00172627	0,00551541	0,00882876	0,00978249
Δ pAi	0,1	6,5309E-47	3,065E-14	1,0301E-09	4,3205E-08	1,8833E-07	2,2731E-07
Δ pAi	0,15	1,1969E-70	2,0716E-27	3,7441E-19	4,6626E-16	6,9719E-15	6,9719E-15
Δ pAi	0,2	3,3252E-95	1,1636E-43	1,6614E-31	8,5632E-27	4,1421E-25	1,9885E-25
Δ pAi	0,25	1,227E-120	1,7683E-62	1,5329E-46	3,1722E-40	8,6765E-39	3,6071E-39
Δ pAi	0,3	5,303E-147	1,526E-83	4,2697E-64	2,5968E-56	3,9328E-54	2,5968E-56
Δ pAi	0,35	2,36E-174	1,184E-106	4,5658E-84	4,7426E-75	4,2624E-73	4,0958E-77
Δ pAi	0,4	9,446E-203	1,082E-131	2,137E-106	1,8014E-96	3,3252E-95	6,725E-102

# aff	affected individuals
# non aff	non affected individuals
pAi non aff	allele frequency in non affected individuals
Δ pAi	% Difference in allele frequency between affected and non-affected individuals

Figure 3 (III)

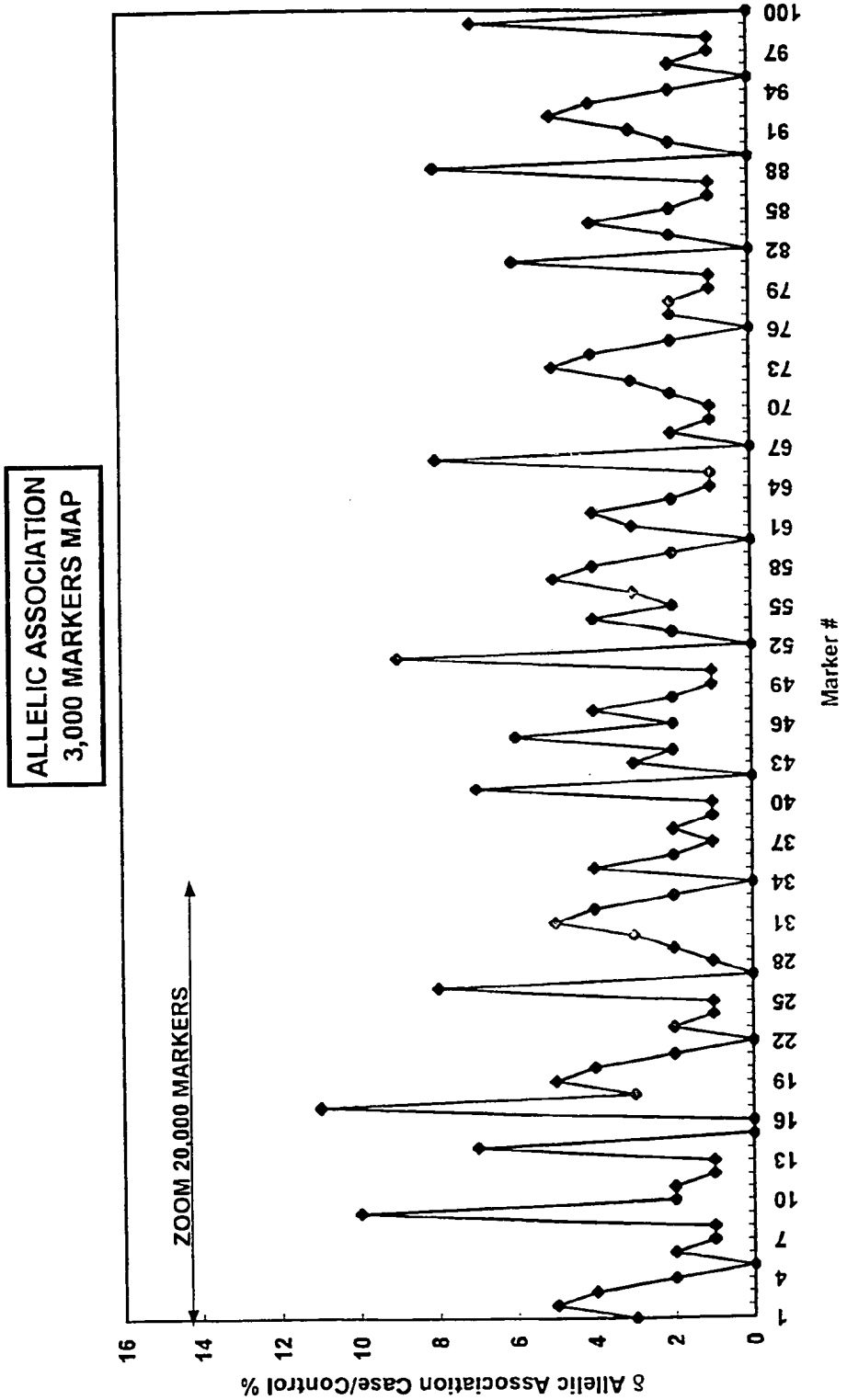

ALLELIC ASSOCIATION
3,000 MARKERS MAP
ZOOM 20,000 MARKERS
δ Allelic Association Case/Control %
Marker #
0
2
4
6
8
10
12
14
16
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
61
64
67
70
73
76
79
82
85
88
91
94
97
100

Figure 4

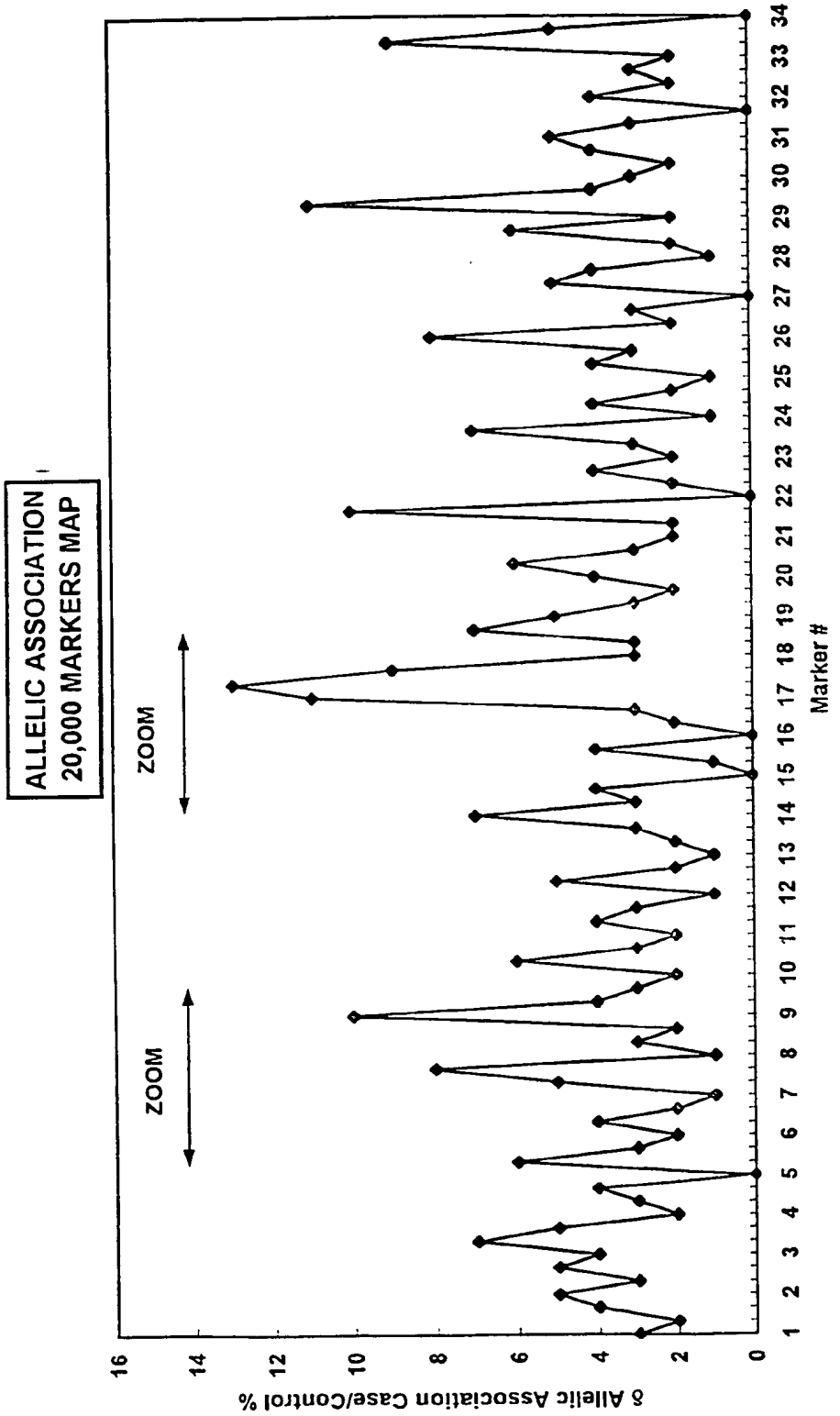

ALLELIC ASSOCIATION
20,000 MARKERS MAP
ZOOM
ZOOM
Marker #
δ Allelic Association Case/Control %
0
2
4
6
8
10
12
14
16
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34

Figure 5

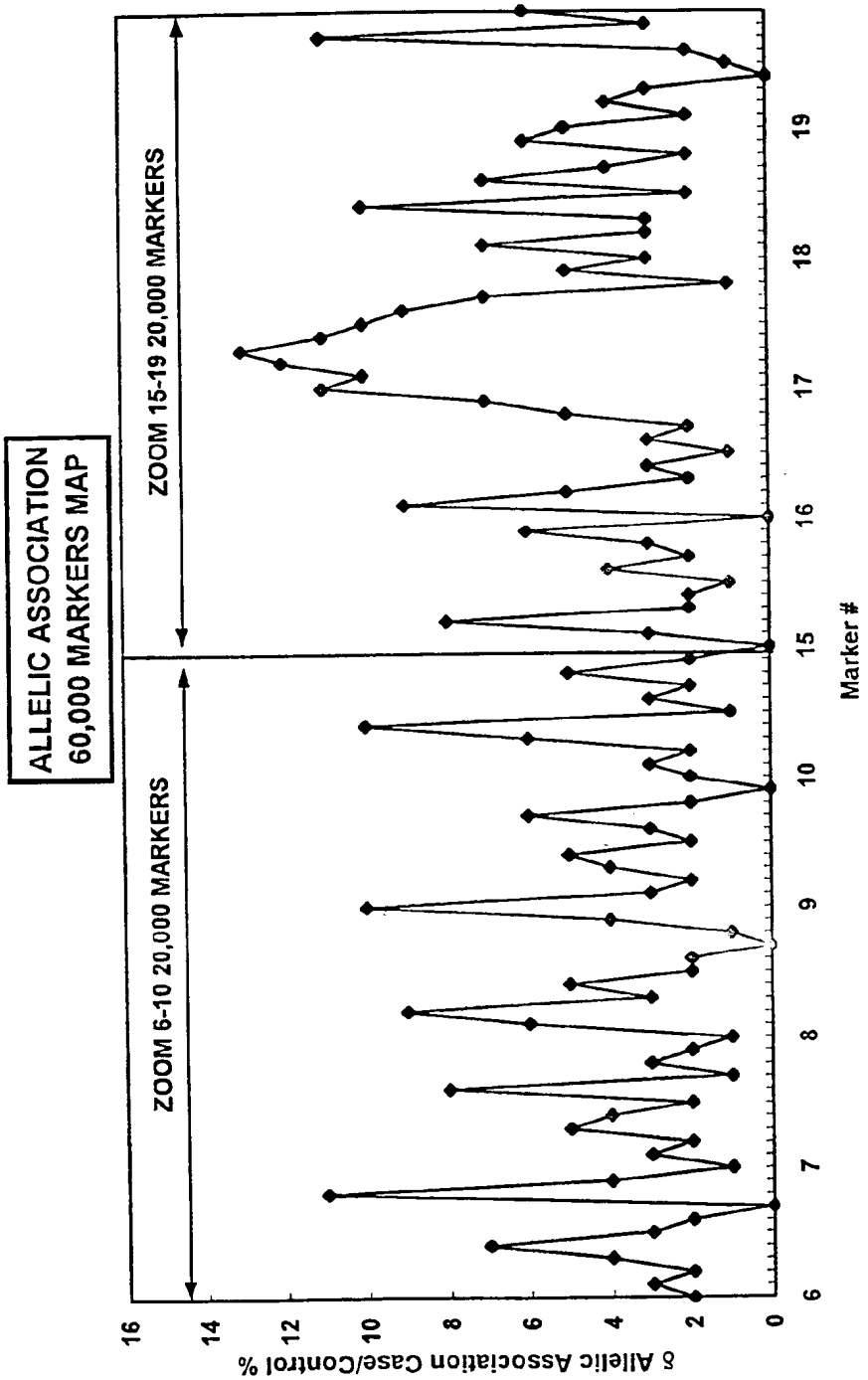

ALLELIC ASSOCIATION
60,000 MARKERS MAP
ZOOM 6-10 20,000 MARKERS
ZOOM 15-19 20,000 MARKERS
δ Allelic Association Case/Control %
Marker #
0
2
4
6
8
10
12
14
16
6
7
8
9
10
15
16
17
18
19

Figure 6

APO E REGION HAPLOTYPE FREQUENCY ANALYSIS

POPULATIONS	AD CASES (225)	AD CONTROLS (248)

markers	99-366	99-344	99-359	99-355	haplotype frequencies		odds-ratio	P value
p value	3,01E-01	1,11E-01	6,63E-01	1,38E-01	cases	controls		
haplotype 1	C	G			0,404	0,308	1,52	**3,05E-03** ***
haplotype 2		G	A		0,203	0,165	1,29	1,24E-01 *
haplotype 3			G	G	0,375	0,306	1,36	2,83E-02 **
haplotype 4	C		A		0,264	0,209	1,36	5,95E-02 **
haplotype 5		G		A	0,115	0,071	1,70	1,64E-02 **
haplotype 6	C			A	0,15	0,129	1,19	3,59E-01 *
haplotype 7	T		G	G	0,225	0,122	2,09	4,76E-05 *****
haplotype 8	**T**	**A**	**G**	**G**	**0,228**	**0,108**	**2,44**	**2,05E-06** ******

Figure 7

APO E REGION HAPLOTYPE SIMULATION
POPULATION : 225 CASES vs 248 CONTROLS

Haplotype 8	4 Markers				haplotype frequencies		odds-ratio	pvalue
	99-344/439	99-366/274	99-359/308	99-355/219	cases	controls		
	A	T	G	G	0,228	0,108	2,44	2,05E-06 *****

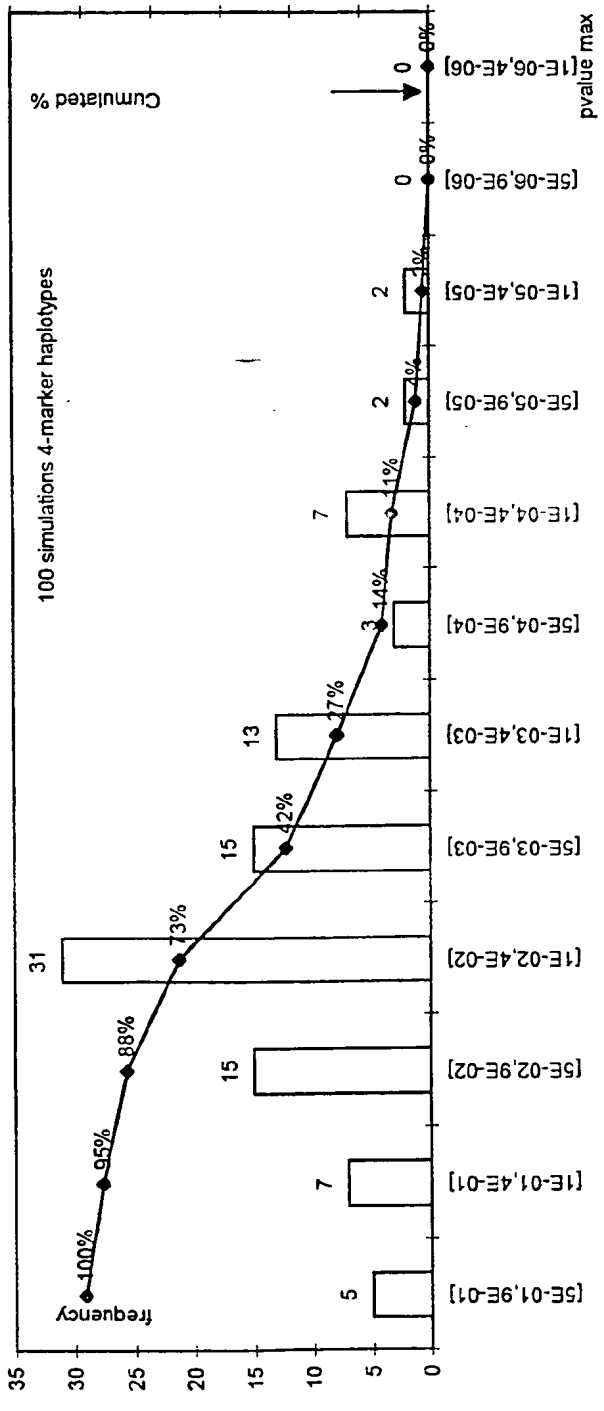


Figure 8

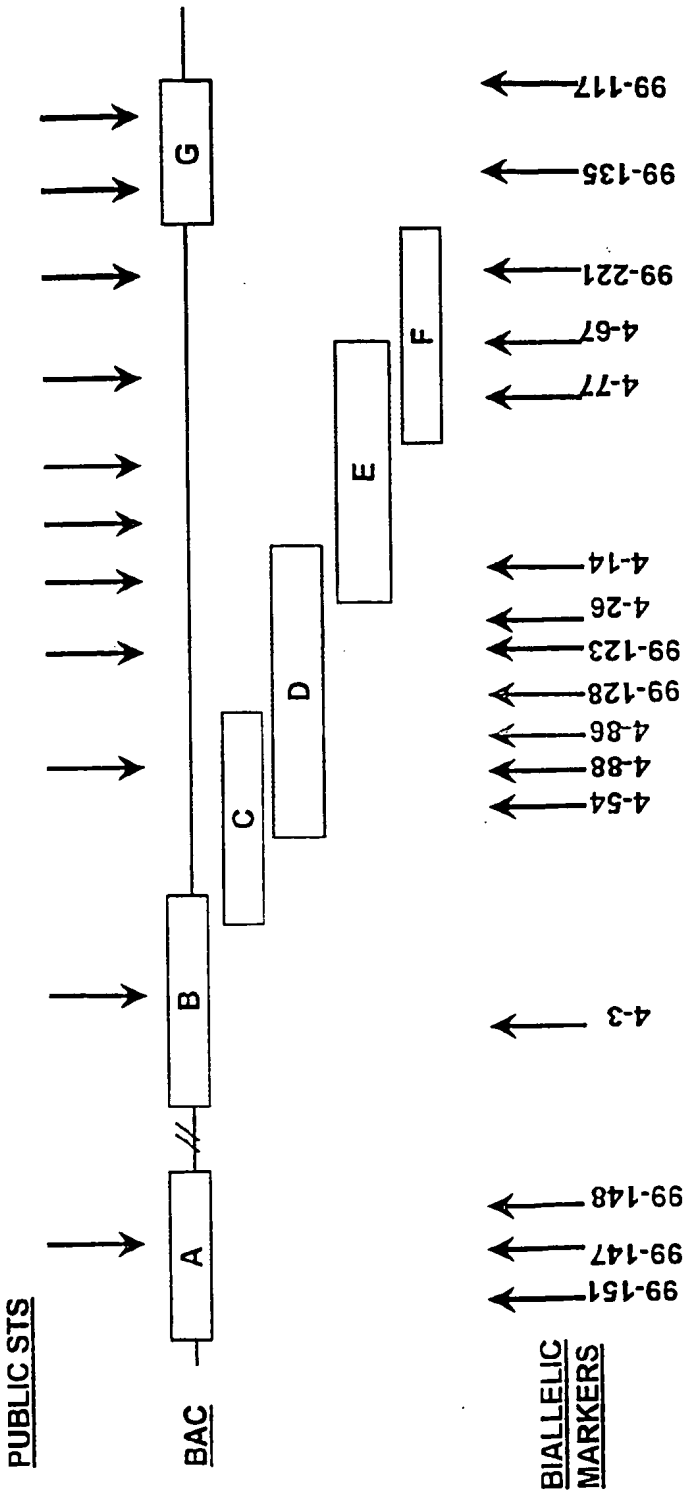

PUBLIC STS
BAC
BIALLELIC MARKERS
A
B
C
D
E
F
G
99-148
99-147
99-151
4-3
4-54
4-88
4-86
99-128
99-123
4-26
4-14
4-77
4-67
99-221
99-135
99-117

Figure 9

PROSTATE CANCER ASSOCIATION STUDIES (FIRST SCREENING)

Population	PROSTATE CANCER	NON AFFECTED
Sample size	CASES = 112	CONTROLS=76
Population Characteristics	35 sporadic cases + 77 familial cases	> 65 years PSA<4

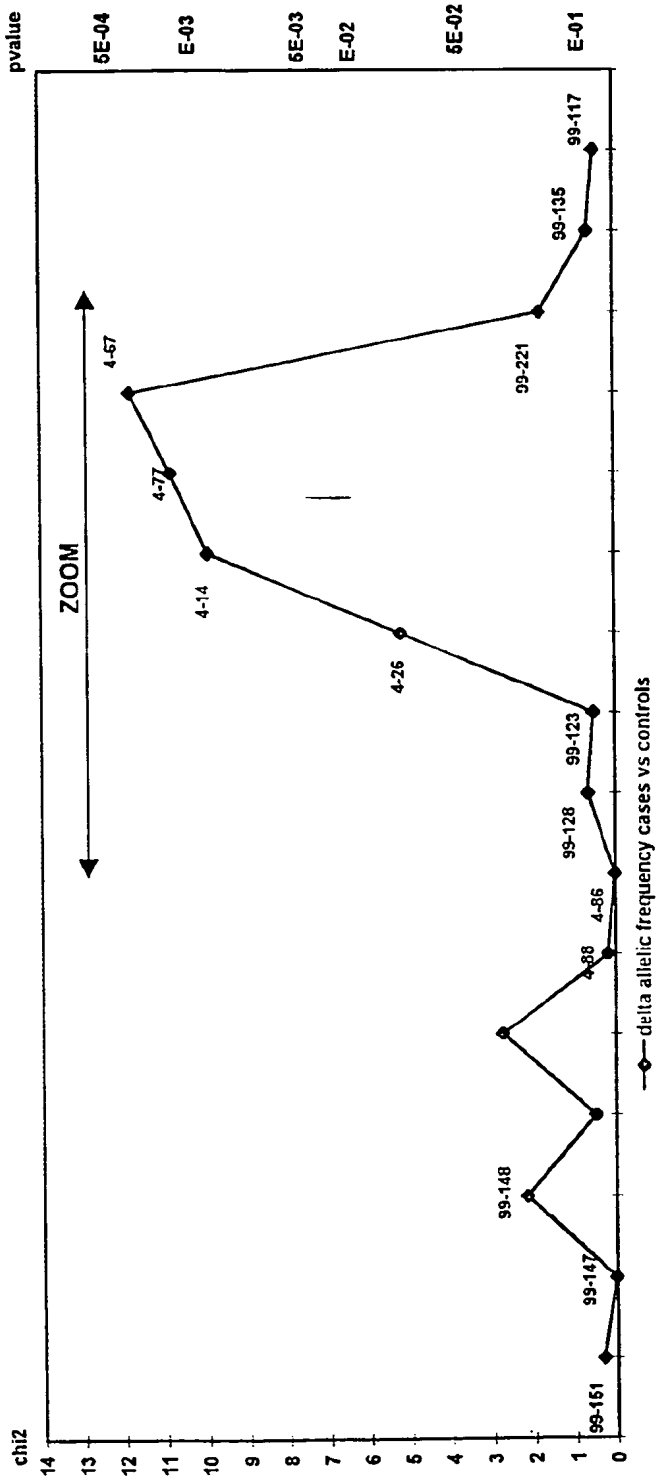


Figure 10

PROSTATE CANCER ASSOCIATION STUDIES (ZOOM)

	PROSTATE CANCER CASES (185)	NON-AFFECTED CONTROLS (104)
characteristics of populations	47 sporadic cases + 138 familial cases	> 65 years PSA<4

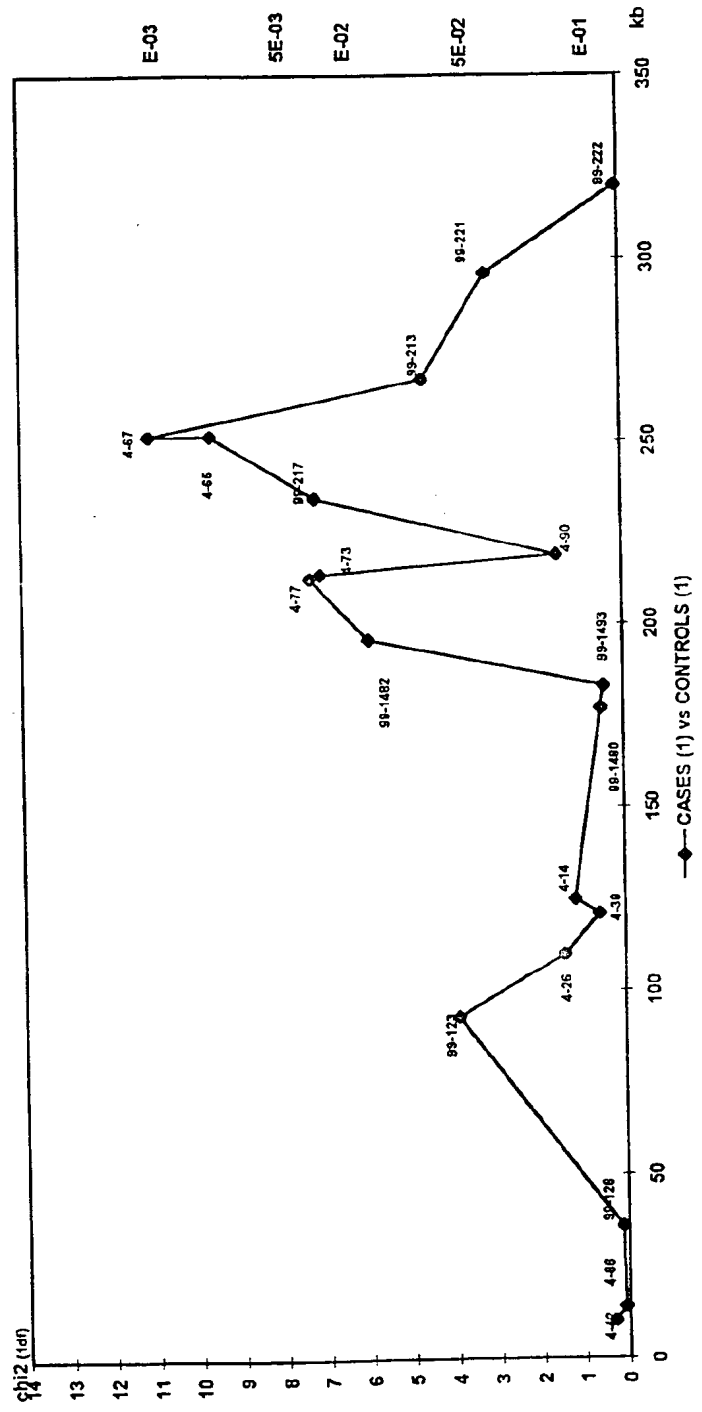


Figure 11

PROSTATE CANCER HAPLOTYPE FREQUENCY ANALYSIS

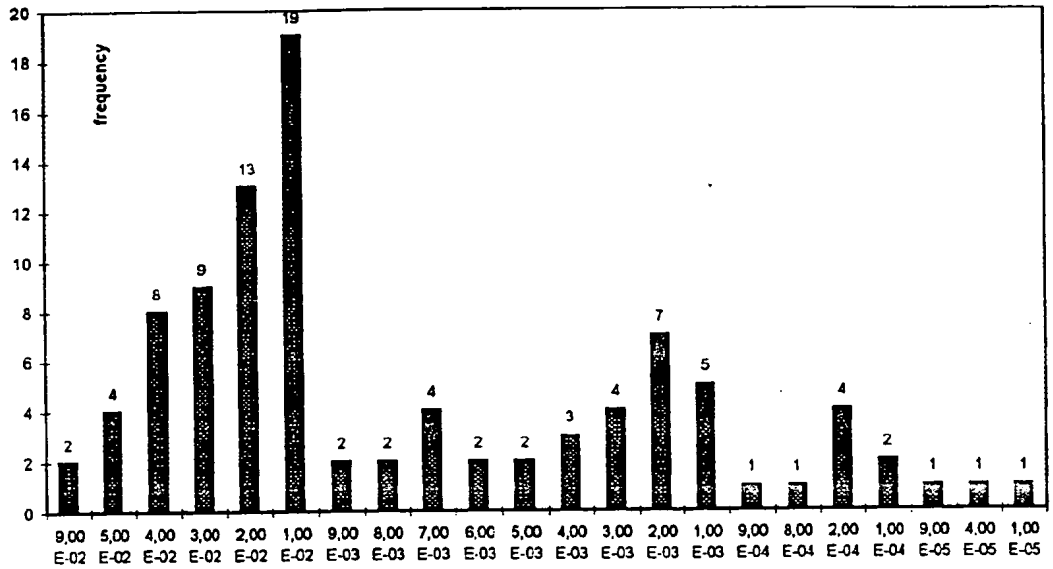
	PROSTATE CANCER CASES (281)	NON-AFFECTED CONTROLS (130)
characteristics of populations	143 sporadic cases + 138 familial cases	> 65 years PSA<4

markers	99-123	4-26	4-14	4-77	99-217	4-67	99-213	99-221	99-135	haplotype frequencies		relative risk	pvalue
bacs	H0287B09	B0189E08		B0463F01					B0725B12				
genes	<——PG1——												
p value	2,00E-01	1,00E-01	1,00E-01	2,00E-02	2,00E-02	6,00E-04	9,00E-02	7,00E-01	2,00E-01	cases	controls		
haplotype 8 >304kb<	C	A	C	G	T	T	C	A	A	0,075	0,018	4,42	9,00E-04 ***
haplotype 7 >286kb<		A	C	G	T	T	C	A	A	0,095	0,016	6,46	6,00E-05 ****
haplotype 6 <186kb>		A	C	G	T	T	C	A		0,116	0,019	6,78	1,00E-05 *****
haplotype 5 <171kb>			C	G	T	T	C	A		0,117	0,013	10,06	9,00E-07 ******
haplotype 4 <83kb>				G	T	T	C	A		0,117	0,025	5,17	2,00E-05 *****
haplotype 3.1 <54kb>					T	T	C	A		0,117	0,027	4,78	2,00E-05 *****
haplotype 3.2 <54kb>				G	T	T	C			0,222	0,109	2,33	4,00E-05 *****
haplotype 2.2 <39kb>				G	T	T				0,251	0,134	2,17	2,00E-04 ****
haplotype 2 <32kb>					T	T	C			0,226	0,112	2,32	1,00E-04 ***
haplotype 1.1 <17 kb>					T	T				0,256	0,146	2,01	3,00E-04 ****
haplotype 1.2 <15 kb>						T	C			0,233	0,129	2,05	6,00E-04 ***

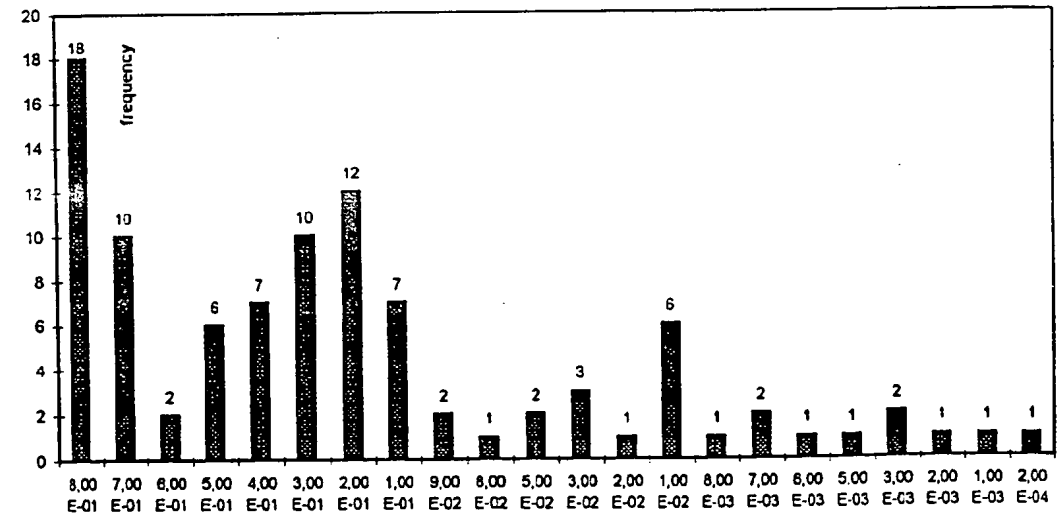
Figure 12

PROSTATE CANCER HAPLOTYPE SIMULATIONS (100 ITERATIONS)

							haplotype frequencies		relative	pvalue
markers	4-14	4-77	99-217	4-67	99-213	99-221	cases	controls	risk	
haplotype	C	G	T	T	G	A	0,117	0,013	10,06	9,00E-07



pvalue max of haplotypes for 100 simulations



pvalue of haplotype CGTTGA for 100 simulations

Figure 13

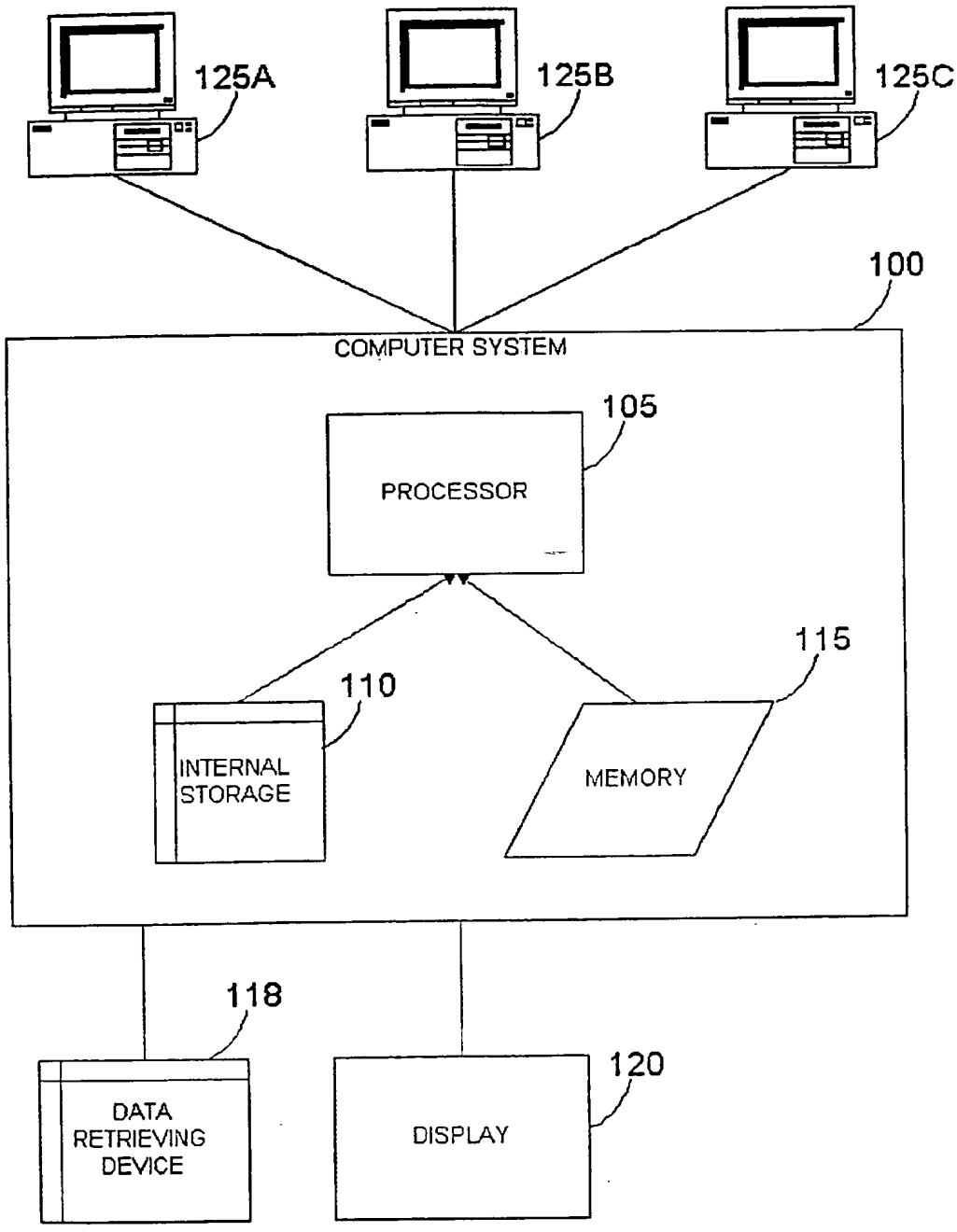

125A
125B
125C
100
COMPUTER SYSTEM
105
PROCESSOR
115
110
INTERNAL STORAGE
MEMORY
118
DATA RETRIEVING DEVICE
120
DISPLAY

Figure 14

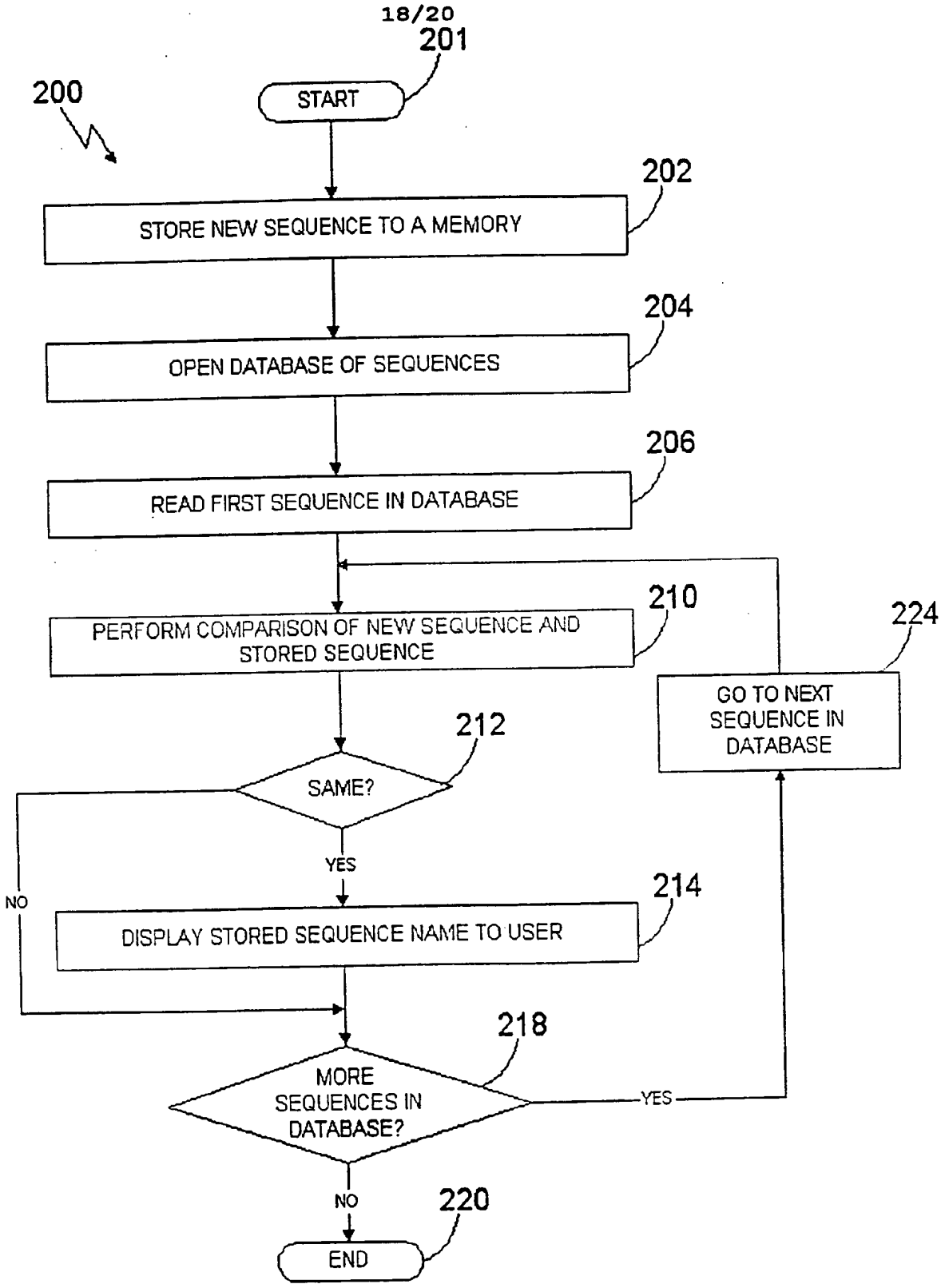

200
201
START
202
STORE NEW SEQUENCE TO A MEMORY
204
OPEN DATABASE OF SEQUENCES
206
READ FIRST SEQUENCE IN DATABASE
210
PERFORM COMPARISON OF NEW SEQUENCE AND STORED SEQUENCE
212
SAME?
YES
NO
214
DISPLAY STORED SEQUENCE NAME TO USER
218
MORE SEQUENCES IN DATABASE?
YES
NO
220
END
224
GO TO NEXT SEQUENCE IN DATABASE

Figure 15

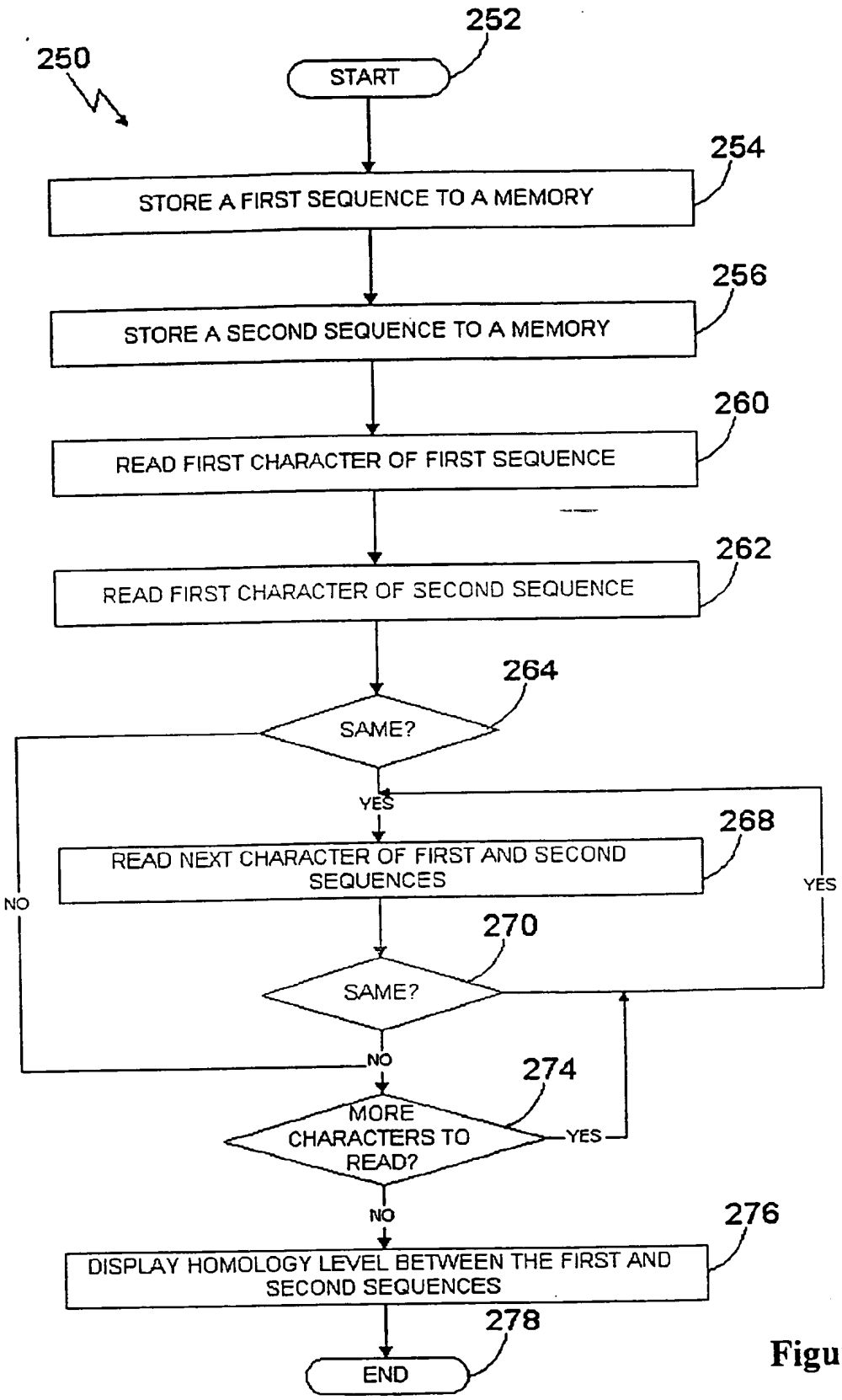

250
252
START
254
STORE A FIRST SEQUENCE TO A MEMORY
256
STORE A SECOND SEQUENCE TO A MEMORY
260
READ FIRST CHARACTER OF FIRST SEQUENCE
262
READ FIRST CHARACTER OF SECOND SEQUENCE
264
SAME?
YES
NO
268
READ NEXT CHARACTER OF FIRST AND SECOND SEQUENCES
YES
270
SAME?
NO
274
MORE CHARACTERS TO READ?
YES
NO
276
DISPLAY HOMOLOGY LEVEL BETWEEN THE FIRST AND SECOND SEQUENCES
278
END

Figure 16

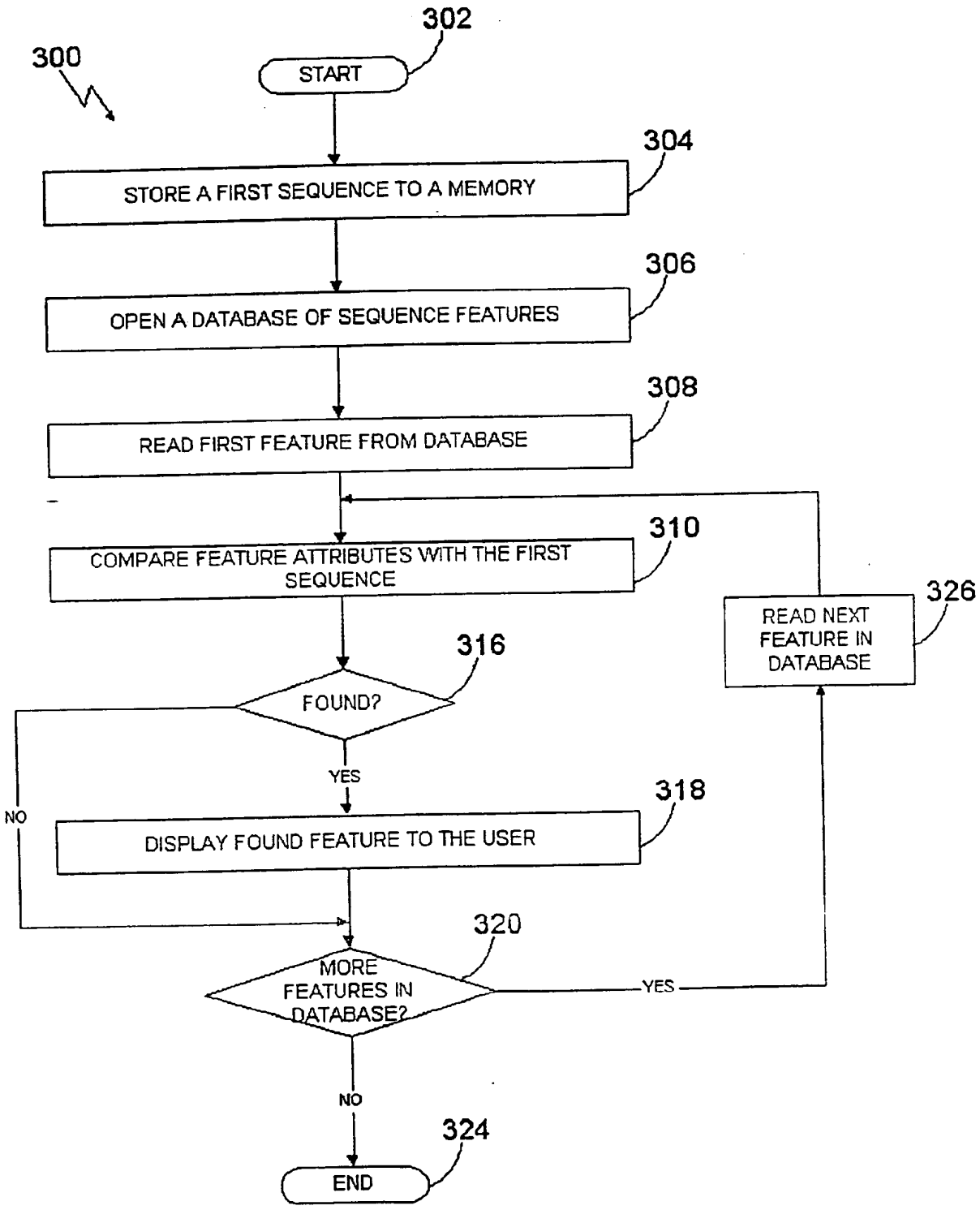

300
302
START
304
STORE A FIRST SEQUENCE TO A MEMORY
306
OPEN A DATABASE OF SEQUENCE FEATURES
308
READ FIRST FEATURE FROM DATABASE
310
COMPARE FEATURE ATTRIBUTES WITH THE FIRST SEQUENCE
316
FOUND?
YES
NO
318
DISPLAY FOUND FEATURE TO THE USER
320
MORE FEATURES IN DATABASE?
YES
NO
324
END
326
READ NEXT FEATURE IN DATABASE

Figure 17